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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **300th Meeting** *of the Biochemical Society was held at the Rowett Research Institute, Bucksburn, Aberdeenshire, on Saturday, 22 September* **1951**, *when the following papers were read:*

COMMUNICATIONS

The Formation of Lactic Acid in Grass-Water Mixtures. By A. J. G. Barnett. (*Division of Agricultural Biochemistry, Department of Biological Chemistry, University of Aberdeen*)

An apparatus has been designed for the study of fermentations in mixtures of minced grass and water. The mixtures are placed in 5 l. insulated and darkened aspirators fitted with tubulures for sampling purposes, and CO_2-free air or N_2 may be passed through the apparatus in order to alter conditions of fermentation. Arrangements are made to collect NH_3 or CO_2 evolved, in concentrated H_2SO_4 and concentrated KOH (10N), while temperature changes within the mass are recorded on thermographs. Normally, the finely minced grass (1400 g.) is mixed with 2 l. of CO_2-free distilled water and the mixture thoroughly stirred before being placed in the aspirator. The grass weight is suitably altered, dependent upon its dry-matter content, to give a final mixture of about 240 g. of dry matter in a total liquid content equivalent in volume to about 3500 ml.

In the present series of experiments attention has been devoted to the formation of lactic acid, and this has been correlated with pH change and the decline in soluble carbohydrate concentration. Lactic acid has been estimated by the adaptation due to Barnett (1951*a*, *b*) of the technique of Barker & Summerson (1941) for lactic acid in muscle, while total soluble carbohydrates have been determined by the anthrone method as used by Barnett & Miller (1950).

The results so far obtained have indicated that within the first 4 days the pH of such mixtures falls from about pH 6 to less than 4, while the lactic acid concentration is produced at maximum rate during the first 8 days, at temperatures of between 18 and 21°. The final concentration of lactic acid is of the order indicated by Virtanen (1937) in silage, but the results are of interest in that they are not in accord with the generally held view that to ensure adequate lactic acid production in silage, relatively dry material should be ensiled and that this should reach a temperature in the fermenting mass of about 37°.

The addition of molasses in suitable concentration to the above mixtures made little difference to the lactic acid formation or to the pH, while the addition of glutamic acid or leucine in small amounts, a procedure suggested by the findings of Dolby & Waters (1944) and Harry (1951) in studies on *Lactobacillus casei*, had a slightly depressing effect on lactic acid production in the mixtures under consideration.

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The Synthesis of Lysine by Bacteria during Incubation of Rumen Contents *in vitro*. By Mary L. McNaught (introduced by E. C. Owen). (*The Hannah Dairy Research Institute, Kirkhill, Ayr*)

Pearson & Smith (1943) and Smith & Baker (1944) have shown that when the liquid portion of rumen contents is incubated with carbohydrate, *in vitro*, bacterial protein is synthesized from non-protein-nitrogen (N.P.N.). They also showed that some proteins were hydrolysed by rumen bacteria whereas others were not. This observation agrees well with the findings of many workers that the amino-acid pattern of dietary protein as judged by its biological value is changed by the rumen organisms (Cuthbertson & Chalmers, 1950; McDonald, 1948). The present communication reports the changes in the lysine content of rumen liquid incubated *in vitro*.

Rumen liquid was prepared from fresh rumen contents as described by Smith & Baker (1944). Before and after incubation, samples of rumen liquid were preserved for total lysine estimations. Further samples were partitioned into filtrates and sedimented by prolonged centrifugation as described by Smith & Baker (1944). Lysine was estimated in all these fractions microbiologically by the method of Barton-Wright (1946). Some difficulty was encountered at first owing to the presence in the hydrolysates of a substance which inhibited the growth of the test organism. This was overcome by evaporating the hydrolysates under reduced pressure to remove HCl, followed by steam distillation, first under slightly acid conditions and then under slightly alkaline conditions.

In one such experiment (Exp. 1) the lysine in the whole rumen liquid increased from 9·3 to 11·6 mg./100 ml., while the lysine in the supernatant liquid decreased from 5·8 to 5·1 mg. and in the sediment increased from 3·6 to 6·5 mg., giving an overall synthesis of 2·2 mg. as compared with 2·3 mg. in the whole liquid. In Exp. 2 there was greater protein synthesis from N.P.N., and it was found that lysine in the whole liquid increased from 11·5 to 18·1 mg./100 ml., in the supernatant from 6·8 to 7·5 mg. and in the sediment from 4·4 to 10·4 mg., giving an overall synthesis of 6·7 mg. as compared with 6·6 mg. in the whole liquid. The amount of lysine in whole rumen contents in Exp. 2 was also estimated, and it was found that the increase of 6·6 mg. lysine which occurred in the rumen liquid represented a 12% increase in the lysine of whole rumen contents.

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The Effect of Mastitis on the Phosphatase and Phosphorylated Esters in Cow's Milk. By R. Chanda, E. C. Owen and P. S. Watts.* (*The Hannah Dairy Research Institute, Kirkhill, Ayr*)

The partitions of phosphorus and aneurin and the concentrations of some other constituents in the milk of a cow suffering from mastitis were determined. Chloride estimations showed that the two left quarters were seriously affected, that the right-fore (R.F.) quarter was apparently normal and that the right-hind quarter was only slightly affected. These changes in the chloride were accompanied by large increases in phosphatase. The phosphatase from the L.F. quarter was four times that from the R.F. quarter. Mastitis therefore affected milk phosphatase and chloride in much the same way. As noted by Rowland (1938), the N content of the milk was increased by the mastitis. In contrast the P content was simultaneously reduced. The ratio of N : P was increased from 4·8 in the normal quarter to 7·0 in the L.F. quarter. This change in the ratio of N : P in mastitis is similar to that found by Chanda & Owen (1951) in cows treated with thiouracil.

The partitions of phosphorus were closely related to phosphatase. The inorganic-P was 75% of the total P in the milk of the worst affected quarter (L.F.) compared with only 54% in that of the normal quarter (R.F.). The ester-P was larger in the normal quarter than in the affected quarters. There was also a big decrease in the lipid-P and casein-P in the affected quarters. The figures for casein-P indicated that the synthesis of casein was impaired by the mastitis.

The ratio of total to free aneurin for the affected quarters approached unity as compared with a value of 3·1 in the normal quarter, and this shift in the ratio occurred in spite of the decrease in the total aneurin due to mastitis. In the milk of small aneurin content there was also a small content of non-lipid solids. When the phosphatase was larger the ratios of total to free aneurin were smaller. Thompson (1945) noted a similar shift in the ratio of total to free aneurin in mastitis milk.

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The Effect of Thyroxine, Thiouracil and Stilboestrol on the Metabolism of Carotene and Vitamin A in Lactating Goats. By R. CHANDA, HELEN M. CLAPHAM and E. C. OWEN. (*The Hannah Dairy Research Institute, Kirkhill, Ayr*)

Thyroxine increases and thiouracil decreases the digestibility of carotene in lactating cows (Chanda, Clapham, McNaught & Owen, 1951; Chanda, McNaught & Owen, 1951). These changes are reflected in the carotene and vitamin A content of the milk (Chanda & Owen, 1951). It has now been found that the digestibility of carotene in goats is similarly affected by these drugs, but that the increase of digestibility of carotene due to thyroxine is larger in the cows than in the goats. By contrast the decrease of digestibility due to thiouracil is larger in the goats than in the cows.

Thyroxine markedly increased the vitamin A content of goat milk and butterfat, while thiouracil caused an opposite but less marked effect. When lactating goats were treated simultaneously with thyroxine and stilboestrol, lactation was inhibited, but before lactation ceased the percentages of fat and of vitamin A in the milk showed large increases. The increase in vitamin A in this case was, however, proportional to the increase in fat so that there was no marked effect on the vitamin A content of the fat itself.

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The Nitrogen Metabolism of *Penicillium notatum*. By R. M. MACKENZIE and R. P. COOK. (*Department of Biochemistry, University College, Dundee*)

Penicillium notatum (Strain 1249 B21) was grown, in surface culture, on a chemically defined medium, based on that of Calam & Hockenhull (1949). Nitrogen was provided as ammonium sulphate, and the concentration for optimal growth of mycelium determined. The mycelium and culture medium were separated, normally after 20 days' growth, and the distribution of the main nitrogen-containing fractions was determined. Paper partition chromatography of the free and combined amino-acids was carried out.

Free amino-acids and a very small amount of protein (cf. Clutterbuck, Lovell & Raistrick, 1932) were found in the culture medium. The mycelium contained mainly free amino-acids and a small amount of non-dialysable nitrogen-containing material, extractable with dilute sodium hydroxide. This material gave a biuret reaction and could be precipitated by means of trichloroacetic acid. It contained 5·7 % nitrogen which could be increased to 13·6 % by treatment with phenol (Morgan & Partridge, 1941).

The general conclusion is drawn that the amount of large molecular weight protein in the mycelium of *P. notatum* is small. During the early stages of growth, glutamic acid followed by valine appear in the culture medium. There is some evidence that the percentage of mycelial nitrogen present as protein is initially quite high, but that it decreases with increasing age of the culture.

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The Changes in Cacao Polyphenolic Substances during Fermentation. By W. G. C. FORSYTH. (*Colonial Microbiological Research Institute, Trinidad, British West Indies*)

Fresh cacao beans are prepared for marketing by heaping in boxes so that their covering of mucilaginous pulp is broken down through the action of yeasts and acetic acid bacteria (Knapp, 1937). The fermented beans are then dried in the sun. During the fermentation and drying various changes take place in the cotyledons which result in a product suitable for the manufacture of chocolate.

In previous work (Forsyth, 1949, 1951; Forsyth & Rombouts, 1951) it has been shown that the soluble

polyphenols of the fresh cacao bean consist mainly of three classes of compounds, catechins, anthocyanins and leucoanthocyanins; and that they can be detected by paper chromatography and isolated by chromatography on columns of cellulose pulp. In fermented, sun-dried beans, all those polyphenols have been destroyed.

By quantitative paper chromatography of extracts of beans removed from the fermentation heap throughout the process the destruction of the main polyphenols has now been followed. In this way the changes in (−)-epicatechin (by far the major catechin present), two leucoanthocyanins and two anthocyanins have been determined. The substances are destroyed at different rates and in a definite order. The order is contrasted with the changes taking place during artificial treatments of the beans, such as grinding in air, in water, in buffers, with enzyme poisons, and under anaerobic conditions. It would appear that the internal fermentation of the cacao bean is essentially an anaerobic process. This is in direct contrast to the prevalent theory, which depends mainly on analogy with tea fermentation, that the change is one of oxidation by a polyphenol oxidase system, dependent on external oxygen. (−)-Epicatechin which would be expected to act as substrate to the oxidase present persists throughout the fermentation period, whereas the simple cyanidin compounds are converted to more complex leucocyanidins. The catechin is only removed during the sun drying. In contrast, over 90 % of the catechin is destroyed by 30 min. grinding of fresh beans in air.

It is suggested that the major change during fermentation is a conversion of the simple anthocyanins to complex leucocyanidins by condensation under anaerobic conditions.

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The Carbohydrates of Human Cerebrospinal Fluid. By Marjorie D. Eastham and K. R. Keay (introduced by R. P. Cook). (*Department of Biochemistry, University College, Dundee*)

The reducing substance in normal cerebro-spinal fluid (c.s.f.) is generally considered to be glucose, but 'a ketose probably fructose' was reported by Hubbard & Russell (1937).

The carbohydrates of 57 normal c.s.f.'s have been investigated by paper partition chromatography (Partridge, 1948), using the following colour reagents: benzidine (Horrocks, 1949); naphthoresorcinol and trichloroacetic acid (Forsyth, 1948); dimethylamino-benzaldehyde and acetylacetone- (Elson & Morgan, 1933). Glucose, fructose and glucosamine are present in the free condition. Total reducing substances (mg./100 ml.) estimated by the method of Hagedorn & Jensen ranged from 66 to 112, mean 88, and by the method of Somogyi (1930) ranged from 35 to 90, mean 67. Fructose estimated by the method of Roe (1934) was 1·6–7·2 mg./100 ml., mean 3·8, and glucosamine estimated by the method of Elson & Morgan (1933) was 6–19 mg./100 ml., mean 11. In a bulk sample the carbohydrates were separated by quantitative chromatography (Flood, Hirst & Jones, 1947). The phenylosazone of glucose and fructose was prepared, as was the *p*-nitrobenzylidene derivative of glucosamine. There is evidence for the occasional existence of other keto-sugars and of pentoses.

Preliminary investigations have been made on the polysaccharides and on their hydrolysis products by the Dische carbazole estimation as used by Seibert & Atno (1946) for blood-serum polysaccharides.

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Observations on the Growth of *Escherichia coli* on Amino-acids as Sole Nitrogen Source. By E. A. DAWES. (*Department of Biochemistry, University of Glasgow*)

The ability of *Escherichia coli* to grow in glucose-phosphate medium containing single amino-acids as sole nitrogen source and its dependence on conditions of aeration has been investigated. Such growth is of interest since it reflects production of the requisite deamination systems.

Growth is good in serine, alanine, cysteine, glutamic and aspartic acid media, but poor with valine, tryptophan, tyrosine, phenylalanine, threonine, methionine and isoleucine. Furthermore, good growth is obtained in aerated media with alanine, serine, aspartic and glutamic acids individually as sole carbon and nitrogen sources. Relationships established between stationary population and alanine or serine concentration demonstrated increased total crops with both these substrates on aeration, despite the fact that alanine deaminase is an aerobic and serine an anaerobic deaminase, and that production of the latter enzyme during growth in peptone medium is stimulated by anaerobic conditions (Stephenson & Gale, 1937, 1938).

The well-known effect of glucose in suppressing deaminase activity of organisms grown in complex media was investigated with the synthetic serine medium. Glucose concentrations of $3{\cdot}98$–$66{\cdot}3 \times 10^{-3}$M were without effect on serine deaminase activity as evidenced by growth rate and stationary population; activity of washed cell suspensions was not increased by addition of the coenzymes adenosine-5-phosphate, glutathione and biotin.

Wood & Gunsalus (1949) presented evidence that serine and threonine deaminases may be one and the same enzyme; in the present work serine supported good growth whereas DL- or L-threonine did not, even if a serine-grown inoculum was used. DL-Threonine causes a lowered growth rate and reduced total crop when added to serine or glucose-ammonium salt medium; in the latter case viable counts established that the growth rate was first increased and then decreased, compatible with the production of a threshold concentration of an inhibitory substance. α-Ketobutyric acid, primary product of threonine deamination, causes both decreased growth rate and stationary population, whereas propionic acid (which may arise from α-ketobutyrate) produces a lag phase, but has no effect on growth rate or stationary population. Comparison of deamination activities of ammonium- and serine-grown cells gave serine : threonine ratios of 21 : 1 and $5{\cdot}5$: 1 respectively. Warburg experiments showed that threonine is not readily oxidized even by cells harvested from threonine media, whereas serine is rapidly oxidized although the oxygen uptake is reduced when threonine is present simultaneously.

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The Combination of Peptides with Metals. 1. Cupric Ions with Glycylglycine and Diglycylglycine. By HAZEL DOBBIE, W. O. KERMACK and H. LEES. (*Department of Biological Chemistry, University of Aberdeen*)

The combination of metals with amino-acids has been quantitatively investigated fairly extensively (Riley & Gallafent, 1931; Borsook & Thimann, 1932; Albert, 1950; Maley & Mellor, 1950; Monk, 1951), but comparatively little work has been directed to the combination of metal ions with polypeptides. In the present work two methods have so far been used: (*a*) the potentiometric titration of mixtures of peptide and copper salt, extensively employed for the amino-acids by Albert (1950), and Maley & Mellor (1950); (*b*) direct estimation of the cupric ion concentration, or rather the activity, by means of the copper electrode. The results so far obtained are consistent with the following interpretations.

Glycylglycine and copper. When one atom of copper and one molecule of glycylglycine are present, Cu^{++} co-ordinates with the N of the NH_3^+ group displacing one proton, stability constant $K_1 = 10^{6{\cdot}1}$. Then a second proton is displaced, presumably from the amide linkage, so as to form a chelate compound. Formally, the process may be represented as the dissociation of an acid with $pK_a = 4{\cdot}50$. In alkaline regions a third proton is apparently lost ($pK_{a'} = 9{\cdot}65$); this seems to indicate the uptake of a hydroxyl group by the copper under these conditions.

When two or more molecules of glycylglycine are present per cupric ion, the chelate compound proceeds to combine with a second molecule of

dipeptide involving the loss of two more protons. The second stability constant K_2 is $10^{3\cdot1}$, whilst for the dissociation of the proton $pK_{a''} = 9\cdot75$.

Diglycylglycine and copper. When one molecule of diglycylglycine is present per cupric ion, three protons are lost in all, presumably one from the NH_3^+ group and one from each of the two peptide bonds. The values of the constants found here are: stability constant $K_1 = 10^{5\cdot4}$, $pK_a = 5\cdot50$, $pK_{a'} = 7\cdot20$. If, however, 2 molecules or more of diglycylglycine are present, per cupric ion, four protons in all are lost. The simplest assumption is that a dichelate system is formed, similar to that postulated for glycylglycine. The second stability constant is $K_2 = 10^{4\cdot9}$ and $pK_{a''} = 8\cdot00$.

If we assume the constants found from the data, the expected titration curves and also the expected copper electrode potential may be calculated. The titration curves agree over the range observed, while the copper electrode potentials show satisfactory agreement as long as the copper concentration exceeds 10^{-8} M.

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The Prosthetic Group of Xanthine Oxidase. By D. B Morell. (*The Molteno Institute, University of Cambridge*)

Corran, Dewan, Gordon & Green (1939) and later workers have shown that xanthine oxidase, in purified concentrated solution, is a reddish enzyme containing flavin-adenine dinucleotide (FAD). This red colour, atypical for flavoproteins, led Corran *et al.* (1939) to postulate a component of the prosthetic group in addition to FAD. This view was supported by the finding that hypoxanthine reduced the enzyme flavine of an apparently highly active preparation only very slowly.

The present experiments show that the FAD of xanthine oxidase is anaerobically reduced by hypoxanthine, xanthine, salicylaldehyde or xanthopterin in two phases: (1) a fast reduction occurring in less than 30 sec. ('immediate' reduction) and (2) a slow reduction which may take 1 hr. or more. Substrates of the enzyme, and dithionite, reduce the optical density of the enzyme at 450 mμ. by the same amount. This is contrary to some earlier reports (Corran *et al.* 1939; Horecker & Heppel, 1949) but in agreement with Ball (1939).

The activity of the enzyme per unit total FAD is proportional to the percentage of enzyme FAD which is 'immediately' reduced. FAD attached to active enzyme protein reduces FAD attached to inactive enzyme protein slowly (second phase, see above). The percentage of active enzyme in any preparation can be determined by the percentage 'immediate' reduction.

A concentration of free FAD approximately equal to that of the FAD attached to active enzyme protein takes 5 min. for reduction anaerobically. The ability of the D-amino-acid oxidase and notatin systems to reduce the free co-enzyme is considerably less. Thus FAD cannot act significantly as a free, mobile, carrier linking other flavine dehydrogenase systems with the above systems in the same way that cozymase links the triosephosphate and lactic dehydrogenase systems in the glycolytic cycle.

The red colour of xanthine oxidase preparations is due to the effect of the FAD-protein linkage in the intact enzyme on the spectrum of the FAD. High concentrations of $CaCl_2$ (and other divalent metal salts) split this linkage and the difference spectrum (untreated − treated enzyme) shows that in the intact enzyme (*a*) the absorption of FAD at 450 mμ. is 67% higher than for free FAD, and (*b*) the greater absorption in the longer wavelengths is responsible for the red colour.

There is thus no real evidence that there is any component of the prosthetic group other than FAD.

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Physical Toxicity in Aqueous Solutions. By J. C. McGowan. (*Imperial Chemical Industries Research Department, Alkali Division, Northwich, Cheshire*)

In the past, evidence has been brought forward to show that a large number of simple organic compounds produce toxic symptoms in living organisms when they reach a definite concentration in some biophase (Ferguson, 1939; Gavaudan, Dodé & Poussel, 1944). The author (McGowan, 1951) has examined this hypothesis for the case of vapours in the light of certain theories of solubility (Uhlig, 1937; Eley, 1939) and has found that the biophase appears to be either identical or very similar in all organisms. Also relationships were found by means of which it was possible to estimate the toxicities of many gases and vapours.

The toxicities of aqueous solutions have now been considered and, for non-associated compounds which do not participate in hydrogen bonding, the equation $-\log_{10} C_t = -\log_{10} C' + k[P]$ has been derived. Here C_t and C' are the concentrations of the toxic agent (g. mol./l.) in water and in the biophase respectively, $[P]$ is its parachor (ml.), and k is a constant. This equation has been found to hold and many examples of its application can be given. The constant k has been found to have the value 0·012, and C' is, of course, constant for any given manifestation of toxicity. Corrections can be applied for the presence in the molecules of the toxic compounds of groupings giving rise to association and hydrogen bonding. The equation is useful for the prediction of toxicities of compounds which would be expected to be physically toxic and for the classification of data on toxicities of aqueous solutions.

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Spectrophotometric Determination of Fructose-1:6-diphosphate, Hexosemonophosphates, Adenosinetriphosphate and Adenosinediphosphate. By E. C. Slater. (*Department of Pharmacology, New York University, and Molteno Institute, University of Cambridge*)

Spectrophotometric methods of measuring those intermediary metabolites which react with either oxidized or reduced pyridine nucleotides have been developed recently, especially by Ochoa and his associates. Racker's (1947) method of estimating hexokinase and phosphohexokinase activity has now been adapted for the determination of phosphorylated sugars and adenine nucleotides. Fructose-1:6-diphosphate (HDP) reacts with an excess of dihydrocozymase (CoH_2) in the presence of a rabbit muscle fraction, according to the following equations:

(1) HDP → glyceraldehydephosphate + dihydroxyacetonephosphate.

(2) Glyceraldehydephosphate → dihydroxyacetonephosphate.

(3) 2 Dihydroxyacetonephosphate + $2CoH_2$ → 2 glycerolphosphate + 2Co.

Overall reaction (A): HDP + $2CoH_2$ → 2 glycerolphosphate + 2Co.

The disappearance of CoH_2, determined by the decrease of optical density at 340 mμ., is a measure of HDP.

If phosphohexokinase, magnesium and excess ATP are added as well as the muscle fraction and CoH_2, glucose-phosphate (GP) and fructose-6-phosphate (FP) will react as follows:

(4) GP → FP.

(5) FP + ATP → HDP + ADP.

(6) ADP → ½ATP + ½ adenylic acid.

(A) HDP + $2CoH_2$ → 2 glycerolphosphate + 2Co.

Overall reaction (B): GP + ½ATP + $2CoH_2$ → 2 glycerolphosphate + 2Co + ½ adenylic acid.

The disappearance of CoH_2 is, in this case, a measure of GP + FP + HDP (GP is the sum of glucose-6-phosphate and glucose-1-phosphate).

If excess GP or FP is used with limited amounts of ATP or ADP, reaction (B) can be used to measure the reactive phosphate groups of ATP and ADP. ATP can be determined separately by the hexokinase reaction (C):

(C) ATP + glucose → GP + ADP.

The reaction is stopped with trichloroacetic acid and GP estimated on the neutralized supernatant by reaction (B). 0·05 μmol. of phosphorylated sugar or reactive phosphate of the adenine nucleotides

can be measured with an accuracy of a few per cent. Pyruvate and oxalacetate are included in HDP and must be separately determined with lactic dehydrogenase. Creatinephosphate is included in ADP, but can be determined separately by adding creatinephosphokinase and ADP to the components of reaction (C). Triosephosphates are included in HDP.

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The Purification of Ox Liver β-Glucuronidase. By EVELYN E. B. SMITH. (*Biochemistry Department, University of Glasgow*)

Cohn, Gurd, Surgenor, Barnes, Brown, Derouaux, Gillespie, Kahnt, Lever, Liu, Mittelman, Mouton, Schmidt, & Uroma (1950), have studied the separation of the protein components of human plasma by means of metallo-protein complexes combined with low-temperature ethanol fractionation. The use of metallic ions has proved advantageous in the purification of ox liver β-glucuronidase.

The method of purification consists essentially of an initial precipitation of inactive protein as a zinc complex, followed by subsequent precipitation of the enzyme at a low acetone concentration. The enzyme precipitate is eluted with a lower acetone concentration containing barium ions, temperature and pH being strictly controlled. By selective fractionation of the acetone-barium eluate with ammonium sulphate, a highly active preparation can be obtained. Controlled dialysis of the latter results in precipitation of the enzyme, which thus appears to be a globulin in nature. On elution of this precipitate with 0·2 M-acetate buffer pH 4·0, an enzyme solution is obtained containing 200,000 units of activity per mg. protein nitrogen (1 glucuronidase unit equals 1 μg. phenolphthalein liberated in 1 hr. from 10^{-3} M-phenolphthalein glucuronide at 37°). The initial homogenate of ox liver contained 6000 units per g. tissue, this representing a 5000-fold purification of the enzyme. On the basis of activity per g. of protein, an 800-fold purification has been achieved.

The properties of the purified enzyme were discussed.

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The Influence of Partial Hepatectomy and Other Procedures on the β-Glucuronidase Activity of Rat Liver. By G. T. MILLS. (*Biochemistry Department, University of Glasgow*)

It was recently shown by Mills, Smith, Stary & Leslie (1950) that, following partial hepatectomy, there was a rise in rat-liver β-glucuronidase which occurred 4 days after operation, but that this rise was probably not related to the degree of cellular proliferation in the liver as suggested by Levvy, Kerr & Campbell (1948) for mice.

A more detailed experiment has now been carried out in which the previous findings have been confirmed. Liver β-glucuronidase activity only shows a rise at 4 days after operation, at which time the rapid phase of cellular proliferation, as indicated by the deoxyribonucleic acid (DNA) content of the liver and protein regeneration, is substantially complete. 4 Days after partial hepatectomy the β-glucuronidase/DNA ratio and β-glucuronidase/protein ratio show significant increases, indicating an increased β-glucuronidase per cell.

Sham-operated animals were used to control this experiment, and here it was found that there was a significant fall in liver β-glucuronidase activity on the basis of liver weight, DNA or protein nitrogen content. The β-glucuronidase activity had returned to normal after 4 days.

Experiments have also been carried out in which liver damage was produced by carbon tetrachloride, injected subcutaneously in arachis oil and controlled by arachis oil injections alone. Both groups of animals showed elevations of liver β-glucuronidase activity, the results being particularly striking in the case of the control group.

From experiments such as these it is difficult to establish any relationship between increased β-glucuronidase activity and cellular proliferation.

An interesting point which has emerged from our work is that there appears to be a strain difference in the liver β-glucuronidase activity of rat liver. This has been shown to exist in the case of mice by Morrow, Greenspan & Carroll (1949, 1950).

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The β-Glucuronidase Activity of Chemically Induced Rat Hepatoma. By G. T. Mills and Evelyn E. B. Smith. (*Biochemistry Department, University of Glasgow*)

Evidence has been produced by Fishman, Anlyan & Gordon (1947), and Fishman & Bigelow (1950), which indicates that in many cases cancer tissue has a higher β-glucuronidase activity than the normal tissue of origin, and it was suggested on the basis of these findings that an elevated β-glucuronidase activity may be characteristic of malignant tumours.

One great difficulty in the interpretation of work on tumours is that of obtaining adequate controls of a comparable cell type. The fact that in many cases the cell type of the tumour is very different from that of the tissue of origin may mean that any changes in β-glucuronidase activity only reflect a change in cell type, without being related to cancerous changes *per se*.

Experiments have been carried out on chemically induced rat hepatoma. The cell distribution in hepatoma is very similar to that of normal liver, the main differences being in the chemical composition of the cell itself.

Hepatomata were induced in rats by feeding for 5 months on the diet described by Griffin, Nye, Noda & Luck (1948), containing 0·06 % *p*-dimethylaminoazobenzene. Control groups were maintained on carcinogen-free diet and on normal laboratory stock diet for the same time.

It has been found that the hepatomata produced by *p*-dimethylaminoazobenzene contain less β-glucuronidase activity than normal liver when expressed in terms of either fresh liver weight, deoxyribonucleic acid content (cf. Mills, Smith, Stary & Leslie, 1950), or protein-N content of the liver. It has also been found that the protein content of the hepatoma cell is less than that of the normal liver cell, and that the β-glucuronidase is not lost at the same rate as other liver proteins in the change from liver to hepatoma cell.

In view of the reduced β-glucuronidase activity of chemically induced rat hepatoma, considerable caution must be exercised in proposing a relationship between raised β-glucuronidase activity and cancerous tissue. It is more than likely that the raised β-glucuronidase activities seen in some tumours are to be related to changes in cell type, and are not characteristic of cancer tissue itself.

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The Multiple Nature and Potency for Different Micro-organisms of the Vitamin B_{12} Activity of Calf Rumen Contents and Faeces. By J. E. Ford, S. K. Kon and J. W. G. Porter. (*National Institute for Research in Dairying, University of Reading*)

Coates, Ford, Harrison, Kon, Porter, Cuthbertson & Pegler (1951) showed that different organisms widely used for the assay of vitamin B_{12} gave markedly different responses to the vitamin B_{12} activity of gut contents and faeces.

In further work calf rumen contents and faeces were extracted in the presence of cyanide, the extract treated with charcoal and the charcoal eluted with hot 75 % acetone. This eluate was concentrated under reduced pressure and then chromatographed as follows:

(1) At 37° by the descending technique on Whatman no. 1 paper strips, using water-saturated *sec.*-butanol in the presence of a trace of cyanide. Application of the strips to plates of seeded *Escherichia coli* basal medium and subsequent incubation showed the presence of three distinct fractions possessing vitamin B_{12} activity for *Esch. coli*. The first fraction (A), R_F 0·13, was slower moving than vitamin B_{12}, the second, R_F 0·29, moved at the same rate as, and was inseparable from, vitamin B_{12}, and the third (B), R_F 0·54,

was faster moving. Bioautographs showed that fraction A was also active for *Lactobacillus leichmannii* and *Euglena gracilis*, though its activity for *Lb. leichmannii* was appreciably less than for *Esch. coli*. Fraction B was inactive both for *Lb. leichmannii* and for *Euglena gracilis*.

(2) On alumina at pH 3. Development with water gave an eluate that when rechromatographed on paper separated into the above three fractions. Further elution of the alumina with 1% sodium bicarbonate gave an eluate that contained fraction A and a trace of vitamin B_{12}. Assays of this sodium bicarbonate eluate again showed that fraction A was considerably less active for *Lb. leichmannii* than for *Esch. coli*.

The discrepancy between results obtained with *Lb. leichmannii* and *Esch. coli* for the vitamin B_{12} activity of gut contents and faeces can thus be satisfactorily explained in terms of the relative vitamin B_{12} potencies of the three fractions.

Work on the isolation and characterization of fractions A and B is continuing.

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Further Observations on Carotenogenesis by *Phycomyces blakesleeanus*. By J. Glover, T. W. Goodwin and W. Lijinsky. (*Department of Biochemistry, University of Liverpool*)

The role of acetate. 4-Day old mycelia were transferred to N_2-free media (total vol. 90 ml.) containing glucose (1% w/v) and [2-^{14}C] acetate (0·165% w/v; 60 μc./mmol.). The β-carotene (300 μg.), extracted 3 days after transference, contained $8{\cdot}4 \times 10^{-2}$ μc. of the activity originally present in the acetate. Thus about 13% of the C-atoms in the carotene molecule originated from the methyl group of acetate; this percentage is increased to about 32 if it is assumed that there is no turnover of β-carotene.

Using a different basal medium, Grob, Poretti, von Muralt & Schopfer (1951) obtained 25% incorporation of [2-^{14}C] acetate into carotene and considered that this confirmed previous claims (Schopfer & Grob, 1950) that acetate is the primary precursor. Assuming that the dissimilation of glucose yields two acetate residues, then in our experiments the effective dilution of the labelled carbon in the acetate available for β-carotene synthesis is 3·44 μc./mmol. C. The observed concentration of radioactive C in the total C of the isolated carotene was 3·78 μc./mmol., indicating no preferential utilization of acetate for carotenogenesis under these conditions. The results of Grob *et al.* (1951) can also be taken to demonstrate that acetate does not appear to enter the carotene molecule as a complete C_2 unit, for when they used [*carboxy*-^{14}C]-acetate twice as many of the C-atoms of carotene were labelled as when [2-^{14}C] acetate was used.

The conclusion that acetate is probably not a specific carotene precursor was strengthened when it was found that mycelia transferred to media containing acetate as carbon source produced only little carotene; furthermore, this was very much less than that produced by mycelia transferred to an equivalent amount of glucose.

Most of the labelled acetate was dissimilated and ^{14}C was found in lipids, protein, carbohydrate and CO_2.

The mechanism of the stimulatory action of leucine. The following scheme was suggested to explain the stimulatory action of leucine (Goodwin & Lijinsky, 1951):

leucine → α-keto*iso*caproic acid → *iso*valeraldehyde → β-methylcrotonaldehyde → carotene.

α-Keto*iso*caproic acid and β-methylcrotonaldehyde are found to be greatly stimulatory, whilst *iso*valeraldehyde, although active, is less effective. β-Methylcrotonic acid is inactive whilst α-hydroxy-*iso*caproic acid is active.

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DEMONSTRATIONS

Procedures for the Fractionation of Hydrolysis Products of Amylose and Starch. By D. L. Mould and R. L. M. Synge. (*The Rowett Research Institute, Bucksburn, Aberdeenshire*)

Further work here and at Uppsala has shown that the separation observed by Synge & Tiselius (1950) depends primarily on electrophoretic fractionation of negatively charged complexes of the polysaccharides with triiodide ions (cf. Gilbert & Marriott, 1948). In the absence of iodine a slight fractionation occurs in dilute agar-agar gels and can be observed by spraying with iodine after the run. This could be due to the 'electrokinetic ultrafiltration' mechanism originally postulated. Other gels having smaller pores give better separations, and collodion gels are particularly promising. Two separate principles for fractionating breakdown products of starch according to molecular weight are thus available, and applications of them will be demonstrated.

For the electrophoretic fractionation of the iodine complexes the apparatus of Svensson & Brattsten (1949) permits continuous operation. Improved regularity of flow and diminished adsorption were obtained by substituting Ballotini beads Grade 12 (Chance Bros. Ltd.) for powdered glass. Working with amylose hydrolysates (Synge & Tiselius, 1950) in 0·04M-sodium acetate buffer (pH 4·8) containing 0·002N-iodine and 0·002M-KI a space appears between pink- and blue-coloured components. Since the amylose hydrolysate presumably contains polysaccharides of all intermediate chain lengths we deduce that there is a difference in the type of iodine complex migrating on either side of the gap.

For electrokinetic fractionation in absence of iodine, strips cut from collodion ultrafiltration membranes (cf. Elford, 1937) soaked in 0·2N-acetic acid are immersed in liquid paraffin (B.P.) except at their ends, which are in contact with 0·2N-acetic acid into which platinum electrodes dip (cf. Cremer & Tiselius, 1950). Amylose hydrolysate dissolved in the anode chamber enters the electronegative strip on applying a potential; subsequently the amylose solution is replaced by 0·2N-acetic acid and under the applied potential 'elution development' continues. The strip is then removed and the fractionation is visualized by spraying with I_2-KI solution. It has not yet been determined whether molecular-sieve action or adsorption (cf. Brooks & Badger, 1950) is primarily responsible for the encouraging separations observed, but both mechanisms should relatively retard larger molecules. The method has so far been practicable only on the ultramicro scale, using in all 1 mg. polysaccharide. Since it is not dependent on complex formation with iodine it should be generally applicable to polysaccharides and perhaps other substances.

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The Synthesis of Hexuronides and Related Compounds. By C. A. Marsh (introduced by G. A. Levvy). (*The Rowett Research Institute, Bucksburn, Aberdeenshire*)

Fernandez-Garcia, Amoros, Blay, Santiago, Soltero-Diaz & Colon (1950) found that 1:2-*iso*propylideneglucose in aqueous solution maintained at neutrality was smoothly converted by passage of oxygen at 70° in the presence of platinum catalyst into *iso*propylideneglucuronic acid. The latter hydrolysed to give glucuronic acid. (Mehltretter, Alexander, Mellies & Rist (1951) have described, apparently independently, an essentially similar preparation of glucuronic acid.) Application of this reaction to hexosides in the simple apparatus demonstrated has been found to give the corresponding hexuronides in 20–55% yield. Compounds so far prepared include (−)-menthyl α-D-glucuronide (Bergmann & Wolff, 1923), (−)-menthyl β-D-glucuronide (identical with the natural product), methyl α-D-galacturonide (Niemann & Link, 1934), methyl β-D-galacturonide (Morell, Baur & Link, 1935) and methyl α-D-mannuronide (Ault, Haworth & Hirst, 1935). The oxidation of methyl β-D-mannoside is under study. These hexuronides are being used in specificity and inhibitor studies of animal β-glucuronidase and similar enzymes.

Oxidation of 1:2-*iso*propylidene-3-methyl-glucose yielded a product which on hydrolysis gave 3-methyl-glucuronic acid (Levene & Meyer, 1924).

Hydrolysis of methyl α-D-mannuronide yielded D-mannurone. These compounds also are being studied as possible inhibitors of β-glucuronidase.

Oxidation of α- and β-glucose-1-phosphate yielded compounds with the properties of α- and β-glucuronic acid-1-phosphate, respectively. These compounds are of particular interest in view of their possible role as precursors in the biosynthesis not only of the urinary glucuronides (cf. Storey, 1950), but also of plant, animal and bacterial polyuronides.

Preliminary experiments suggest that it may be possible to replace gaseous oxygen by hydrogen peroxide in these reactions.

Gifts of α- and β-glucose-1-phosphate from Dr W. J. Whelan are gratefully acknowledged.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **301st Meeting** *of the Biochemical Society was held in the London Hospital Medical College, Whitechapel, London, E.1, on Friday, 19 October* 1951, *when the following papers were read:*

COMMUNICATIONS

Colorimetric Determination of the β-Glucuronidase Activity of Locust Crop Fluid using 8-Glucuronosidoquinoline as Substrate.

By D. Robinson, J. N. Smith and R. T. Williams. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W.* 2)

8-Glucuronosidoquinoline (Brahm, 1899) is easily obtained crystalline by feeding rabbits with 8-hydroxyquinoline and acidifying the urine with acetic acid. A solution of this glucuronide in water shows a greenish-blue fluorescence in ultraviolet light, whereas 8-hydroxyquinoline itself shows none.

8-Hydroxyquinoline couples readily at pH 4·5–6·5 with Brentamine Fast Blue B Salt (diazotized di-*o*-anisidine; Imperial Chemical Industries Ltd.) to give a purple colour. No colour is given by the glucuronide. Amounts of 4–30 μg. of 8-hydroxyquinoline in 4 ml. solution can thus be easily estimated.

For the estimation of β-glucuronidase activity the enzyme solution was incubated with a $3{\cdot}5 \times 10^{-4}$ M solution of the glucuronide in 0·33 M-acetate buffer for an hour at 37°. The incubated solution was then treated with a 0·1% solution of Brentamine Fast Blue in 0·01 M-potassium acid saccharate. This concentration of saccharate completely inhibits the enzyme. The colour obtained is stable for at least 0·5 hr. and reaches its maximum in 5 min. at 37° or in 15 min. at room temperature. The intensity of the colour was measured in the Spekker absorptiometer.

Pooled crop fluid (1 ml.) from *Locusta migratoria* liberated 64,000 μg. of 8-hydroxyquinoline in 1 hr. at pH 4·5. Locust saliva thus appears to be a potent source of β-glucuronidase. The enzyme was inhibited by saccharate and showed a single pH optimum at 4·5.

Estimations were also made earlier by the method of Spencer & Williams (1951) using $1{\cdot}5 \times 10^{-2}$ M-*p*-chlorophenylglucuronide, the *p*-chlorophenol liberated being estimated spectrophotometrically as its anion at 298 mμ. In this case the estimations were carried out at pH 5·2 which is not the optimum of the enzyme. Nevertheless, 1 ml. of the crop fluid of *Schistocerca gregaria* liberated 10,000–30,000 μg. of *p*-chlorophenol in 1 hr. at 37° and that of *Locusta migratoria*, 15,000 μg.

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Biochemistry of Fluoroacetate Poisoning. Isolation of a Crystalline Inhibitor of Citrate Metabolism.

By R. A. Peters, R. W. Wakelin and P. Buffa. (*The Departments of Biochemistry, Oxford and Istituto di Patologia Generale, Rome*)

The isolation of tricarboxylic acid fractions containing fluorine and inhibiting citrate metabolism has been described recently (Buffa, Peters & Wakelin, 1951); these were synthesized by enzymic action from the kidney homogenates of guinea pigs and oxen poisoned with fluoroacetate. Larger quantities have now been obtained, using especially rabbit and dog kidneys; these have been purified further by the removal of phosphate with propanol-ammonia-water, and the separation of the citric acid by the use of Amberlite exchange resin, I.R. 400 A. After extraction with ether, crystals separated in small amounts; of these as little as 1·24 μg. will inhibit the disappearance of over 4 μmol. citrate in our latest test system. This is the equivalent of 2 μmol. in the test used by Buffa *et al.* (1951). The crystals have been found to contain fluorine in an amount corresponding to one atom of fluorine for one molecule of a fluorotricarboxylic acid by Gillieson & Newcombe (see the following communication). The substance behaves by paper chromatography as a tricarboxylic acid. It is not

yet certain whether it is fluorocitric or fluoro*iso*-citric acid; no colour is given with sodium sulphide in the Pucher, Sherman & Vickery (1936) test for citric acid. It is interesting that the fluorine contents and the weights actually found to be inhibitory are rather close to those reached upon the more impure fractions by inference.

This isolation proves the hypothesis advanced independently by Liébecq & Peters (1949) and by Martius (1949). and taken further *in vivo* by Buffa & Peters (1949) that fluoroacetate inhibits citrate metabolism because it is built into a fluorotricarboxylic acid which jams the tricarboxylic acid cycle. It is now known that the inhibitor inhibits aconitase (Lotspeich, Peters & Wilson, 1951).

We are grateful to Dr Stanford Moore for advice and to Prof. Vernoni for facilities provided.

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Direct Spectrochemical Micro-estimation of Organically Bound Fluorine Using the Hollow-cathode. By A. H. C. P. Gillieson and R. A. Newcombe (introduced by R. A. Peters). (*Atomic Energy Research Establishment, Harwell, Didcot, Berks*)

The method and apparatus used are those described for the estimation of micro quantities of inorganic fluorine by Birks (1950), which is a development of the original apparatus and method of Rand McNally, Harrison & Rowe (1947).

The organic fluorine compound deposited by evaporation of its aqueous solution on the interior surface of a stainless steel cup forming the cathode of the discharge tube was decomposed and the fluorine present excited by positive-ion bombardment by the helium gas through which a discharge was passed.

The spectrum of the discharge was photographed on pre-sensitized Kodak II-N plates using a Hilger Medium spectrograph, and the intensities of the fluorine lines at 7128·0 and 7202·4 A. read by a microphotometer.

For the estimation of the fluorine content of fluorocitric acid, standards of sodium fluoroacetate were used so that conditions of liberation of the fluorine were as similar as possible.

The standard deviation of estimation of 3 μg. of fluorine as sodium fluoroacetate was 16·6%.

Two portions of fluorocitric acid each of 46 μg. were used to obtain a result for the fluorine content of $7{\cdot}7 \pm 1{\cdot}1$% (calc. for monofluorocitric acid, 9·05%).

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Naturally Occurring L-Pipecolinic Acid. By R. I. Morrison. (*Macaulay Institute for Soil Research, Aberdeen*)

Piperidine-2-carboxylic acid, otherwise known as pipecolic or pipecolinic acid, was originally prepared by Ladenburg (1891) by the reduction of picolinic acid with sodium and ethanol. It was resolved by Mende (1896) by the use of the optically active tartaric acids. Willstätter (1901) obtained a partially racemized L-pipecolinic acid by the oxidation of conhydrine, but there is no record of its natural occurrence.

A substance, believed to be L-pipecolinic acid, has now been isolated from an ethanol extract of the leaves of white clover (*Trifolium repens*). It was first detected in paper chromatograms of such an extract as an unidentified ninhydrin-reacting sub-

stance with an R_F value in most solvents somewhat higher than proline. The colour with ninhydrin was a distinctive deep violet-blue which faded rather rapidly.

The substance was separated in a chromatographically pure condition from the other amino-acids in clover leaves, by displacement chromatography on a cation-exchange resin (Amberlite IR-100), followed by chromatography on a column of powdered cellulose. It was recrystallized from solution in 95% ethanol by addition of an equal volume of ether. From 500 g. of dried clover leaves 0·510 g. of recrystallized substance was obtained.

The chromatographically pure substance formed colourless microcrystals, very soluble in water, slightly soluble in absolute ethanol, and almost insoluble in acetone or ether, m.p. 271° (decomp.). (Found: C, 54·8; H, 8·5; N, 10·2; $C_6H_{11}O_2N$ requires C, 55·8; H, 8·5; N, 10·9%) $[\alpha]_D^{18°} - 25·4°$ ($c = 5$, water). It formed an ethanol-soluble copper salt, and could be sublimed *in vacuo* at 200°, almost without decomposition, yielding fine fibrous needle crystals.

The solubility, properties and melting-point were in good agreement with those given in the literature for L-pipecolinic acid (Mende, 1896). The optical rotatory power was rather lower, suggesting partial racemization.

A specimen of DL-pipecolinic acid, prepared by the reduction of picolinic acid with sodium and amyl alcohol (Clemo & Ramage, 1931), was chromatographically indistinguishable from the isolated substance, giving the same distinctive colour with ninhydrin and having the same R_F values in all solvents tested. A specimen of DL-nipecotinic acid, prepared similarly from nicotinic acid, yielded the same colour with ninhydrin, but had somewhat lower R_F values.

Pipecolinic acid has been detected chromatographically in extracts of other plants (*Trifolium pratense*, *Laburnum vulgare*, *Lathyrus*, *Allium ursinum*), and in a soil sample. An unidentified chromatographic fraction obtained from agenized wheat flour (Campbell, Work & Mellanby, 1951) is probably the same substance; and an unidentified spot on paper chromatograms of amino-acids from certain algae (Fowden, 1951) may also be attributable to pipecolinic acid. This amino-acid may therefore be of fairly common occurrence as a plant constituent.

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The Inhibition of β-Glucuronidase by Derivatives of Saccharic Acid. By G. A. LEVVY. (*Rowett Research Institute, Bucksburn, Aberdeenshire*)

β-Glucuronidase is strongly inhibited *in vitro* by saccharate (Karunairatnam & Levvy, 1949). In the hope of obtaining an inhibitor effective *in vivo*, 3-methylsaccharo-1:4-lactone (Levene & Meyer, 1922) was prepared and converted in solution into 3-methylsaccharate. The latter did not inhibit glucuronidase *in vitro*, but the lactone was nearly as effective as saccharate. This suggested that inhibition of the enzyme by saccharate solutions may be due to the presence of saccharo-1:4-lactone. The mixed monolactone of saccharic acid was prepared, and the two constituents were partially separated (Smith, 1944). A fairly pure specimen of the 3:6-lactone caused only slight inhibition of glucuronidase, whilst samples containing the 1:4-lactone, although grossly contaminated with the other, were more powerful than saccharate.

To inhibit β-glucuronidase, it would appear on present evidence that a compound must bear a general structural resemblance to a β-D-glucuronide, but the presence of a carboxyl group at $C_{(6)}$ seems to be more important than the actual size of the ring involving $C_{(1)}$ (cf. Spencer & Williams, 1951).

The author is indebted to Prof. F. Smith for seeding crystals of the two lactones of saccharic acid, and to Mr A. Hay for technical assistance.

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Some Applications of a New Method for the Synthesis of Hexuronides. By G. A. LEVVY, C. A. MARSH and P. G. WALKER. (*Rowett Research Institute, Bucksburn, Aberdeenshire*)

It has been found that the method described by Fernandez-Garcia, Amoros, Blay, Santiago, Soltero-Diaz & Colon (1950) for the preparation of glucuronic acid can be used to oxidize hexosides to hexuronides, making available a series of compounds which, if known, have hitherto been difficult to prepare. These compounds have been examined for their effects on the liberation of phenolphthalein from phenolphthalein glucuronide by mouse-liver β-glucuronidase. Neither methyl α-D-galacturonide, methyl β-D-galacturonide nor methyl α-D-mannuronide had any effect. Menthyl α-D-glucuronide in high concentration slightly activated the enzyme (cf. Masamune, 1934). Since an alternative substrate would have caused a fall in the rate of hydrolysis of phenolphthalein glucuronide, these experiments help to provide badly needed information regarding the specificity of β-glucuronidase. Both α- and β-glucuronic acid 1-phosphate caused an appreciable fall in the hydrolysis of phenolphthalein glucuronide. This finding is open to a variety of explanations.

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Phospholipin Metabolism in the Lactating Mammary Gland of the Rabbit. By G. A. GARTON* and G. POPJÁK. (*National Institute for Medical Research, Mill Hill, London, N.W. 7*)

Two rabbits, rendered slightly lipaemic by continuous cholesterol feeding (cf. Popják, 1946), received intravenous injections of ^{32}P-labelled orthophosphate. The plasma of these animals, containing ^{32}P-labelled phospholipins, was injected into a lactating rabbit. Two hours later the animal was killed and samples of liver and mammary gland taken for P fractionation and determination of the specific activity of each fraction. The following groups of P compounds were examined: inorganic phosphate, alkali-labile phosphorus, alkali-stable phosphorus and phospholipins. The phosphorus content of each was determined according to Allen (1940).

The results showed that, of the mammary gland phospholipins, 4·3% were derived from blood phospholipins in 2 hr., the corresponding figure for liver being 5·2%. About 20% of the mammary gland phospholipins were degraded in 2 hr., thus at least this amount must also have been synthesized. Radioactive phospholipins were isolated from the milk of this animal.

In other experiments, lactating rabbits were injected intravenously with ^{32}P-labelled orthophosphate and similar fractionations of the P-containing compounds of liver and mammary gland carried out. In addition, inorganic phosphate and phospholipins of plasma and milk were examined. These experiments showed the specific activity of the phospholipin P of the mammary gland and liver to be 10·3 and 7·0%, respectively, of the specific activity of the alkali-stable P (precursor glycerophosphate) in one rabbit and 13·8 and 12·05%, respectively, in a second animal. Radioactive phospholipins were again isolated from the milk.

The results obtained will be discussed in relation to the arterio-venous studies of Graham, Jones & Kay (1936) and the work of Aten & Hevesey (1938) on milk-fat formation in the goat.

Further work on the nature and turnover of mammary gland phospholipins is in progress.

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Non-available Water in Precipitates Formed by Antigens and Antibodies. By J. R. MARRACK, R. A. GRANT and R. G. S. JOHNS. (*London Hospital Medical College, London, E.* 1)

According to the lattice theory, antigens and antibodies combine to form networks in three dimensions. Unless the molecules fit closely, these networks must contain a considerable proportion of water into which large molecules may be unable to penetrate. The concentrations of haemoglobin in a precipitate and in a solution in equilibrium with it were measured. The concentration per ml. of water was considerably lower in the precipitate than in the solution.

The Effect of Dioxan on the Formation of Precipitates by Antigens and Antibodies. By R. A. GRANT. (*London Hospital Medical College, London, E.*1)

Dioxan reduces the dielectric constant of aqueous solutions, but in concentrations up to 25% it does not precipitate proteins. When 20% (v/v) of dioxan is present, antibodies and antigens do not form precipitates. In lower concentrations the amount of precipitate is reduced. If a solution of antibody is mixed with dioxan and the dioxan dialysed away, the antibody will then form a precipitate with antigen. However, if antigen and antibody are mixed in the presence of dioxan, no precipitate forms when the dioxan is dialysed away. The relation of these observations to theories of specific precipitate formation were discussed.

Estimation of Glucosamine in the Presence of Protein. By R. G. S. JOHNS and J. R. MARRACK. (*London Hospital Medical College, London, E.* 1)

The standard method of estimating hexosamines is that of Elson & Morgan (1933). It has been shown, however, that under certain conditions this method gives erroneously high values (see, for example, Horowitz, Ikowa & Fling, 1950). We have studied the estimation of hexosamines in the presence of large amounts of γ-globulin; in particular, of glucosamine in immunochemical precipitates. In such systems we find that the observed glucosamine values are approximately 10% greater than the true values; we have not so far been able to eliminate or reduce this error.

Bendich, Kabat & Bezer (1946) have estimated the amount of glucosamine, in precipitates formed with antisera, as a method of testing the completeness of precipitation of the polysaccharide in various preparations of blood group substances by antisera. He made corrections for the apparent glucosamine in the protein of the precipitates and for the solubility of the precipitates; but not for the additive effect of protein that we have observed.

We have tried the method using two preparations of one of these substances (H-substance) and rabbit antisera supplied by Dr W. T. J. Morgan. We have subtracted 10% from the apparent glucosamine to allow for the effect of protein. It seems that there may be further sources of error which we have not detected, for when the antibody was in excess, the calculated recovery of the H-substances added was over 100%.

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Transfructosidation by a Yeast Invertase Preparation. By J. S. D. Bacon. (*Department of Biochemistry, University of Sheffield*)

A yeast invertase preparation (British Drug Houses Ltd. 'Invertase Concentrate') was incubated at 22° in acetate buffer (pH 5·0) with 15% (w/v) sucrose and 25% (v/v) methanol. Examination of the products by paper-partition chromatography revealed the presence of a substance with an R_F value greater than that of free fructose, both in butanol-acetic acid and in phenol. The spot was non-reducing and reacted with the phloroglucinol spraying reagent for ketoses (Horrocks & Manning, 1949).

This substance has been partially purified, by chromatography with aqueous *n*-butanol on cellulose powder (Hough, Jones & Wadman, 1949), and with water on charcoal (British Drug Houses Ltd. 'Activated')—Celite no. 535 (Johns Manville and Co. Ltd.) mixture (Whistler & Durso, 1950), and obtained in the form of a colourless syrup containing no other carbohydrates detectable on paper chromatograms by the use of the benzidine-trichloroacetic acid and phloroglucinol sprays. It was identified tentatively as β-methylfructofuranoside, because: (1) it was non-reducing, (2) it was stable to alkaline hydrolysis, (3) after 30 min. in 0·5% oxalic acid at 100°, hydrolysis was complete, the only sugar detected was fructose, and (4) the reducing sugar liberated was equivalent to the ketose present, (5) it had an alkoxyl content (Pregl, 1945), calculated as methoxyl, of 16·0% ($C_7H_{14}O_6$ requires OMe, 16·0%), (6) its $[\alpha]_D$ in water, calculated on the basis of its ketose content, assuming it to be a methylfructoside, was $-50{\cdot}4°$ (cf. $-52 \pm 2°$, calculated by Purves & Hudson, 1934), (7) it had the same R_F value in butanol-acetic acid as that component of the γ-methylfructoside mixture which is hydrolysed by yeast invertase (cf. Purves & Hudson, 1934).

The rates of hydrolysis by yeast invertase under comparable conditions of a 4% sucrose solution and of a 2% solution of the unknown substance, were as 8·5:1. Purves & Hudson (1934) found rates in the ratio 13·5:1 for sucrose and the invertase-labile component of γ-methylfructoside.

The formation of this substance, and of analogous compounds with ethanol, *n*-propanol and benzyl alcohol, supports the suggestions made by Fischer, Kohtès & Fellig (1951) and Edelman & Bacon (1951) to explain the formation of oligosaccharides during the action of the enzyme on sucrose (Blanchard & Albon, 1950; Bacon & Edelman, 1950), namely, that yeast invertase is a fructose-transferring enzyme, and that its hydrolytic action on sucrose is only one aspect of its transferring activity.

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Bicarbonate Ion and Muscle Potassium. By R. Creese. (*Department of Physiology, London Hospital Medical College, London, E.* 1)

Rat-diaphragm muscles in bicarbonate-free Krebs saline at pH 7·5 lose some 22% of their potassium, and show a low twitch tension (cf. Creese, 1949). When bicarbonate-saline is restored at pH 7·5, the muscle potassium rises to normal values and the twitch tension is more than doubled. Possibly bicarbonate ions exert their effect on muscular contraction by means of a Donnan-type equilibrium with potassium (cf. Conway & Fearon, 1944).

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Chemistry of Connective Tissue Fibrinoid in Rheumatic Fever. By R. Consden, L. E. Glynn and Winifred M. Stanier. (*Canadian Memorial Red Cross Hospital, Taplow, Maidenhead, Berks*)

A characteristic feature of subcutaneous nodules in rheumatic fever is the central core of eosinophil, fibrillar material, usually termed 'fibrinoid' from its histological resemblance to fibrin. Preliminary results of a chemical examination of such material are reported here.

Freshly excised subcutaneous elbow nodules from various patients were each separated as far as possible from adjacent, apparently normal, connective tissue, and were then washed with water, dried and extracted with fat solvents. Collagen was removed by autoclaving with water. The residues and extracts were hydrolysed and two-dimensional paper chromatography was carried out on the hydrolysates (collidine/*o*-cresol). When sufficient fibrinoid became available (from one case), the pooled nodules were analysed for hydroxyproline, tyrosine, tryptophan and reducing sugar (liberated on mild acid hydrolysis). Parallel treatments and analyses were carried out on normal subcutaneous elbow tissue.

In general, the fibrinoid left more residue after autoclaving (25–40 %) than normal tissue (10–15 %). The chromatograms of fibrinoid and normal extracts were typical of collagen. The normal residue chromatograms were similar to that of elastin, but the fibrinoid residue chromatograms were more complex, and suggested the presence of a non-collagen protein, in addition to elastin.

The following quantitative data were obtained for fibrinoid residue, fibrinoid extract, normal residue and normal extract respectively (amino-acid N as percentage total N). Hydroxyproline: 0·7, 7·6, 1·1, 8·2; tryptophan: 0·8, 0, −, 0; tyrosine: 3·4, 1·6, 2·5, 0·7; reducing sugar (%): 2·7, 2·9, −, 1·3. The hydroxyproline and tryptophan figures suggest that the fibrinoid and normal extracts consist mainly of collagen (cf. Bowes, 1951; Neuman & Logan, 1950), that normal residue consists mainly of elastin (cf. Neuman & Logan, 1950), and that the fibrinoid residue contains 50–70 % of elastin. Tyrosine content, however, is high in all cases, except possibly in the normal extract, compared with values reported for animal connective tissue (cf. Bowes, 1951). The polysaccharide content also appears to be higher in both fibrinoid fractions than that reported for skin collagen (Bowes, 1951), or found by us in human Achilles tendon.

Assuming that fibrin is a constituent of fibrinoid, the tryptophan content in the fibrinoid residue corresponds to a maximum of about 30 % fibrin, but this figure is probably much lower in view of the high tyrosine content and the other analytical data.

It is concluded that fibrinoid, in addition to collagen, elastin, and possibly fibrin, contains material rich in tyrosine and polysaccharide.

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Thiocyanates in Milk and Other Biological Fluids. By F. Wokes, P. Wedgwood and J. Wyatt. (*Ovaltine Research Laboratories, King's Langley, and Eastern Gas Board Laboratories, Watford and St Albans*)

Comparison has been made of the pyridine-benzidine method of Aldridge (1945) with the pyridine-pyrazolone method of Epstein (1947) and of Boxer & Rickards (1951) for the determination of thiocyanate in milk and other biological fluids.

Spectrophotometric studies have shown that, during the development of the colour given by pyridine-benzidine, changes occur in its absorption spectrum, the initial maximum at about 500 mμ. being gradually replaced by a maximum at about 545 mμ. due to thiocyanate. To obtain more accurate results, measurements should be made at 545 mμ. until the maximal extinction is obtained, usually in 30–40 min. Such changes do not occur in the absorption spectrum of the colour given by pyridine-pyrazolone, the maximum remaining at about 635 mμ. as compared with 628–630 mμ. for the corresponding maximum with cyanide (Wokes & Willimott, 1951).

Both methods showed thiocyanate to be a regular constituent of cow's milk in amounts ranging from 0·1 to 10 p.p.m., tests being made on over a hundred samples taken directly from pedigree Jersey cows (from the Ovaltine Dairy Farm) and from milk as

normally consumed by the public. The thiocyanate content gradually decreases in milk stored in the refrigerator, but may still be measured after several days.

Similar results were obtained on human milk samples. Thiocyanate was also determined in saliva, urine and semen.

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I. Acetoin and 2:3-Butylene Glycol Formation by Washed Cells of *Aerobacter aerogenes*. II. The Enzymic Formation of Acetoin by preparations from (*a*) *Aerobacter aerogenes*, (*b*) Animal Tissues. By C. P. SPENCER, F. C. HAPPOLD, R. P. HULLIN and R. L. NOBLE. (*Department of Biochemistry, School of Medicine, University of Leeds*)

The Formation of Acetoin and 2:3-Butylene Glycol in the Decerebrate Cat. By J. DAWSON and R. P. HULLIN. (*Department of Biochemistry, School of Medicine, University of Leeds*)

The Enzymic Phosphorylation of D-Glucosamine. By P. T. GRANT and C. LONG. (*Department of Biological Chemistry, Marischal College, Aberdeen*)

Harpur & Quastel (1949) observed the formation of acid groups (CO_2 evolution) when a bicarbonate-buffered ox-brain extract was incubated under anaerobic conditions with D-glucosamine, adenosinetriphosphate (ATP) and Mg^{++}; they attributed this effect to the phosphorylation of glucosamine by ATP, catalysed by brain hexokinase, although no direct evidence for this reaction was obtained. We have now carried out experiments which show directly that D-glucosamine can undergo phosphorylation in the presence of a partially purified yeast hexokinase preparation.

The reaction medium contained the following components (final concentrations in brackets): D-glucosamine HCl (0·0012 M), K-ATP (0·005 M), $MgCl_2$ (0·005 M), KF (0·05 M), potassium phosphate buffer, pH 7·5 (0·04 M). The incubation was carried out at 30° in open tubes, and other conditions were similar to those employed by Long (1951) for the assay of rat-tissue hexokinase. A suitable quantity of baker's yeast hexokinase, partially purified by an adaptation of the method of Bailey & Webb (1948), was added at zero time; after 10 min., enzyme action was stopped by deproteinizing either with 10% trichloroacetic acid (TCA) or with 0·3 N-$Ba(OH)_2$ followed by 5% $ZnSO_4$ (Somogyi, 1945). Glucosamine was determined in the Somogyi filtrates by copper reduction using the reagent of Nelson (1944); ATP was estimated in TCA filtrates as 10 min. hydrolysable P (at 100° in N-acid).

The following were the principal results obtained. (1) Free glucosamine did not disappear from the reaction mixture unless ATP was present; ATP was dephosphorylated only in the presence of glucosamine or other phosphate acceptor such as glucose. (2) The molar rates of disappearance of glucosamine and ATP were very similar. (3) Glucosamine was phosphorylated about 70% as rapidly as glucose under comparable conditions; an equimolar mixture of glucose and glucosamine was phosphorylated at an intermediate rate, suggesting that the two substrates were competing for a single enzyme. (4) In an experiment carried out on a slightly larger scale, the product of glucosamine phosphorylation was isolated by a barium fractionation procedure. Its analysis was consistent with a monophosphoglucosamine structure. This ester is stable to N-acid at 100°, possesses reducing properties and is believed to contain a free $—NH_2$ group.

The location of the phosphate group has not yet been ascertained.

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The Biosynthesis of L-Cystine and L-Methionine Labelled with ^{35}S. By R. B. WILLIAMS and R. M. C. DAWSON. (*Neuropsychiatric Research Centre, Whitchurch Hospital, Cardiff*)

The rapid synthesis of protein occurring during the growth of *Saccharomyces cerevisiae* has been used to prepare the naturally occurring isomers of cystine and methionine labelled with radioactive sulphur (^{35}S). The yeast was grown in a synthetic culture medium containing labelled SO_4^{++} ions as the sole source of sulphur. With vigorous aeration during the period of growth yields of 3·0–3·5 g. of dry lipid-free yeast residue per litre of medium were consistently obtained. During the acid hydrolysis of the yeast residue there occurred a considerable destruction of the liberated cystine. This destruction of labelled cystine was largely blocked by conducting the hydrolysis in the presence of an excess of inactive L-cystine. The yield of isolated cystine was found to be very greatly improved by a preliminary extraction of the yeast residue with 5% (w/v) trichloroacetic acid solution at 90° to remove nucleic acids (Schneider, 1945). The time of the hydrolysis was kept short (4·5–6 hr.) to avoid undue racemization of the cystine. Labelled L-cystine was isolated from the hydrolysate by precipitation at the isoelectric point followed by recrystallization.

Radioactive L-methionine was prepared from the mother liquor of the cystine precipitation by the addition of inactive L-methionine and the isolation of the amino-acid from the hydrolysate by the usual mercury precipitation procedure (Hill & Robson, 1934). Difficulty was experienced in obtaining the product free from a very small amount of cystine which passed through the mercury-acetate precipitation and subsequent purification of the methionine via the mercuric chloride complex. This was reduced to insignificance as a radioactive contaminant by reducing the specific activity of the cystine in the original mother liquor by the repeated addition of inactive cystine hydrochloride and the precipitation of cystine at the isoelectric point. The methods used for measuring the purity of the isolated amino-acids and their freedom from traces of highly radioactive contaminants were described.

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DEMONSTRATIONS

The Detection of Chromatogram Spots by Means of Ultraviolet Radiation. By G. H. BEAVEN, E. R. HOLIDAY and E. A. JOHNSON. (*Spectrographic Unit, Medical Research Council, London Hospital Medical College, London, E.* 1)

A Simple Automatic Fraction Collector for Preparative Chromatography. By J. EDELMAN and R. V. MARTIN. (*Research Institute of Plant Physiology, Imperial College, London, S.W.* 7)

The apparatus consists essentially of a number of glass bulbs connected in series, each of which contains a float designed to prevent significant diffusion between the bulbs when these are filled with liquid. It has the following advantages. (1) It can be constructed rapidly from glass and rubber tubing only, and little more than an elementary skill in glass-blowing is required. The apparatus is thus easily replicated, enabling separations to be carried out on several columns simultaneously. Variation in the volume of the bulbs is easily kept within 10%. (2) Absence of mechanical parts eliminates flooding or failure to fractionate due to mechanical faults. No external source of power is needed. (3) Development of the column automatically stops after the last fraction has been collected. Effluent is then not uselessly directed into an overflow beaker. (4) Rate of development may be increased simply by attaching the apparatus to a filter pump.

The bulbs are connected in a vertical series with rubber tubing. Effluent from the column passes through the series filling the lowest bulb first. The tubes connecting the bulbs must be wide enough to allow the simultaneous passage of liquid and air in opposite directions. The air ultimately escapes by a tube extending above the liquid surface in the reservoir feeding the column. As each bulb fills, the float rises and seals off the tube connecting it to the next unit. Liquid is thus continually passing through the unfilled units, but this has not been

found disadvantageous as they are continually washed with later effluent. When the topmost unit is full, liquid rises in the air-escape tube until it reaches the level of the liquid surface in the reservoir and development of the column ceases. Before the units are separated, the connecting rubber tubes are clamped with screw clips.

A simple device enables collection of fractions from the same column to proceed successively in two or more parallel series of bulbs, thus eliminating the necessity of a long vertical series when a large number of fractions is required.

Although, unlike more intricate fraction collectors designed primarily for accurate quantitative chromatography (e.g. Stein & Moore, 1948; Hough, Jones & Wadman, 1949; Harris, 1951 Grant & Stitch, 1951) the apparatus is not suited to the collection of small (< about 20 ml.) uniform volumes, this is not a disadvantage in preparative chromatography.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **302nd Meeting** *of the Biochemical Society was held in the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.* 1, *on Saturday,* 10 *November* 1951, *and took the form of a Symposium on 'Metabolism and Function of Nervous Tissue'.*

COMMUNICATIONS

Carbohydrate Metabolism of Nervous Tissues. By R. V. Coxon. (*Department of Biochemistry, University of Oxford*)

Evidence regarding the metabolic fate of glucose in brain was reviewed in relation to its storage and breakdown by aerobic and anaerobic reactions; in particular, the applicability of the Embden-Meyerhof fermentative scheme to cerebral cells was discussed in some detail. The behaviour of pyruvate in brain was described with special reference to the nature of its 'active' 2-carbon derivative, the function of aneurin, the operation of the tricarboxylic acid cycle and coupled phosphorylation. The interpretation of experiments utilizing different types of tissue preparations from the nervous system was discussed and an attempt was made to assess the results of such experiments in relation to the physiology of the intact brain and to certain neurophysiological phenomena. The regulation of some of the processes of cerebral metabolism by endocrine and other chemical factors was briefly considered.

Glutamic Acid and its Relation to the Nervous System. By H. Weil-Malherbe. (*Runwell Hospital, Wickford, Essex*)

In a recent review (Weil-Malherbe, 1950) it was suggested that the three reactions of glutamic acid which were then known to occur in brain, viz. oxidative deamination, transamination and amidation, are components of an integrated system whose function is the rapid de-ionization of intracellular NH_4^+. Meanwhile a fourth reaction, the decarboxylation of glutamic acid to γ-aminobutyric acid, has been demonstrated in brain. Glutamine and γ-aminobutyric acid occur in brain in equimolar concentration. Whether this fact be due to a coincidence or not, it points to a major function of glutamic decarboxylase in brain metabolism.

Glutamic acid cannot replace glucose as substrate for the resynthesis of high-energy phosphate bonds in brain slices or for their response to electrical stimulation. On the other hand, it seems to increase the efficiency of other processes observed in brain slices, e.g. the uptake of oxygen, and the transport of K^+ and of amino-acids across the cell membrane. These effects may be due, in an as yet unexplained manner, to the formation of a hypothetical glutamyl phosphate. Evidence is accumulating which suggests that the glutamine amide bond, which from the point of view of energy level is similar to the peptide bond, is involved in the synthesis of peptide chains through 'transpeptidation' ('transamidation') reactions, perhaps with glutathione functioning as an intermediary catalyst. It is also becoming apparent that the number of transamination reactions, at least in heart, liver and kidney, is less restricted than was formerly believed.

In view of the ready availability and the presumably high rate of synthesis of glutamic acid in the brain cell under normal conditions, it is difficult to accept the popular belief that the clinical effects of glutamic acid are due to its action as a 'brain food'. It has previously been concluded (Weil-Malherbe, 1950) that the clinical effects are probably unconnected with whatever special function glutamic acid may have in brain metabolism, but due to an unspecific adrenergic action. The increase of sympathomimetic amines in blood after the intravenous injection of glutamic acid during hypoglycaemic coma has now been confirmed by a new, sensitive and specific fluorimetric method.

The recent literature on the controversial effects of glutamic acid on convulsive disorders, intelligence and behaviour was reviewed. Other topics, such as the relation of methionine sulphoximine poisoning (agene poisoning) to glutamine, and the level of glutamic and α-ketoglutaric acids in the blood of psychotics were briefly mentioned.

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Phosphates and Nucleotides of the Central Nervous System. By H. McIlwain. (*Biochemical Laboratories, Institute of Psychiatry, Maudsley Hospital, London, S.E. 5*)

Chemical events were here presented within the framework of a physiological analysis, proceeding from changes in the whole animal to those in separated systems.

(1) *In vivo*. (*a*) Rapid changes were described linking reactions in energy-rich phosphates with increased functional activity. (*b*) An imposed, prolonged change of function can cause changes especially in polynucleotides. Conversely, change in functioning can be induced by the specific depletion in nucleotides or phosphates which follow some of the B-vitamin deficiencies.

(2) Partially separated or perfused preparations have been relatively little studied.

(3) Separated tissues from the central nervous system: unstimulated, these can provide excellent systems for following the maintenance of phosphates and nucleotides by the tissue, and their considerable autonomy is illustrated by their susceptibility to electrical stimulation. Stimulated, such preparations enable the effects of many agents on phosphates and nucleotides and on excitability to be correlated.

(4) Cell-free systems with particulate elements have yielded important data on oxidative phosphorylation. An interpretation of this was presented which makes understandable the adjustment of the level of carbohydrate metabolism to the functional activity of the brain.

(5) Individual enzyme reactions, the necessary basis of metabolic interpretations of the preceding data, have in some cases been investigated and can show unexpected niceties of adjustment. Creatine phosphokinase, adenosinetriphosphate-consuming reactions, and changes in nucleotides were described. Histochemical work can often be regarded as at this level of biochemical study, but it provides also a wealth of anatomical and cytological detail, which emphasizes the structural complexity within which the biochemical events associated with functional activity in the brain take place.

Lipids of the Central Nervous System. By G. H. Sloane Stanley. (*Biochemical Laboratories, Institute of Psychiatry, Maudsley Hospital, London, S.E. 5*)

The central nervous system (C.N.S.) contains a higher proportion of lipid than any other non-adipose tissue; in fact lipids form its largest single kind of component. The C.N.S. is also unique in the composition of its mixture of lipids; the proportion of cerebrosides and sphingomyelins is unusually high, while neutral fat and cholesterol esters are nearly absent. These lipids, moreover, are almost unaffected by circumstances such as choline deficiency, which produce extreme changes in the lipids of other organs.

Obviously then, the lipids must have vital functions in the C.N.S., as is, indeed, shown by the close connexion between pathological changes in their distribution and abnormal behaviour of the animal.

Their function was assumed, until recently, to be purely structural; partly because of the constancy of their composition, and partly because in early tracer experiments, the administration of abnormal fatty acids or radioactive phosphate had almost no effects on the brain lipids of fully grown animals. Besides, the known lipolytic enzymes only showed very low activities in the C.N.S.

Recently, however, tracer experiments using deuterium (in heavy water), and studies of the metabolism of radioactive phosphate within the C.N.S. itself, have shown that even in adults there is a significantly rapid turnover of some of these lipids. It has also been found that certain brain preparations can break down phospholipins at easily measurable rates; in particular, one of the kephalins is hydrolysed at a speed comparable with that of brain respiration.

Although, therefore, it is still probable that many of the lipids in the C.N.S. form only static (but very important) parts of its structure, there is also now reason to suppose that there may be a direct connexion between the metabolism of certain lipids and nervous and mental activity.

Changes in Brain Constituents in Central Activity. By D. Richter. (*Neuropsychiatric Research Centre, Whitchurch Hospital, Cardiff*)

A continuous expenditure of energy is required to maintain the brain in the state of irritability required for consciousness and for the activity of the lower centres. The brain is never entirely inactive during life, and it must be regarded as functionally active in the resting subject under basal conditions. The energy expenditure is indicated by the oxygen and glucose utilized and by the temperature, which is appreciably higher than that of the arterial blood in the conscious brain.

The energy requirement of the brain is increased in convulsions and decreased in anaesthesia. This is reflected by characteristic changes in the metabolites such as the phosphate esters, which are related to the mechanisms of energy exchange. Similar changes can also be observed to occur under physiological conditions in emotional excitement and in sleep. Of the metabolites which may be related more specifically to nervous activity, change in the brain acetylcholine content and in the phospholipin metabolism are of interest. Evidence for the changes with functional activity has been obtained by the method of rapid freezing with liquid air and by radioactive tracer methods. The ultra-violet micro-spectrophotometer has been used to show changes in the nucleoproteins due to fatigue.

Biochemical Aspects of the Transport of Ions by Nervous Tissue. By R. E. Davies and H. A. Krebs. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Sheffield*)

Potassium and sodium ions can move both with and against concentration gradients in brain and nervous tissue, and the precise linkage between this and energy-giving reactions is one of the outstanding problems in this field.

Guinea pig brain slices and ox retina incubated *in vitro* show a rapid initial loss into the suspending medium of about 50 % of the intracellular potassium, but in the presence of oxygen, glucose and glutamate, the lost potassium is quantitatively recovered. Sodium migrates in a direction opposite to that of potassium in approximately equivalent quantities. There is also an approximate equivalence between the absorption of potassium and glutamate. After the recovery period the potassium concentration of brain remains constant, but there is a continuous rapid exchange with external potassium ions. This may be an 'active' absorption and a 'passive' leakage, or perhaps an example of exchange diffusion, both recovery and turnover require glutamate and the energy from glucose oxidation. Toluene-*p*-sulphonamide, a powerful inhibitor of carbonic anhydrase, reduces the rate of potassium accumulation and causes a fall of the potassium concentration.

A theory of the mechanism of ion accumulation was discussed in which the basic processes are similar to those in gastric mucosa (Davies & Ogston, 1950). The energy from glucose oxidation leads to a separation in space of hydrogen ions, a process involving phosphate-bond energy and a hydrogen carrier, possibly the glutamate-iminoglutarate system. The movements of potassium, sodium and glutamate ions then depend on exchanges with hydrogen ions and, when the rates are high, carbonic anhydrase is required to maintain the intracellular pH.

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The Use of Isotopes in the Study of the Metabolism of the Nervous System. By R. M. C. Dawson. (*Neuropsychiatric Research Centre, Whitechurch Hospital, Cardiff*)

Use of the isotopic tracer technique has shown that many substances and ions penetrate into the nervous system at an extremely slow rate. From studies with the labelled phosphate ion it would seem that ionic exchange between the blood and the brain takes place via the cerebrospinal fluid (C.S.F.). The delay in the entry of phosphate ions into the brain is caused by their slow secretion into the C.S.F. by the choroid plexuses. Once the phosphate has passed into the C.S.F. it rapidly enters the brain substance and exchanges with the labile phosphate groups of phosphocreatine and adenosinetriphosphate, and is then slowly incorporated into the phospholipin and nucleoprotein fractions. If increased radioactivity is found in any particular phosphorus fraction it is not correct to assume an increased synthesis until the results have been correlated with the activity of the precursor of the fraction.

These considerations were applied in discussing the results of investigations which have shown that certain physiological factors influence isotopic exchange and metabolism in the nervous system.

FORTHCOMING PAPERS

It is hoped to publish the following papers in the next issue of the *Biochemical Journal*:

The localization of amine oxidase in the liver cell. By JOYCE HAWKINS

The branched-chain fatty acids of ox fat. 1. The isolation from ox suet of a C_{17} branched-chain saturated fatty acid. By R. P. HANSEN, F. B. SHORLAND and N. J. COOKE

The fate of certain organic acids and amides in the rabbit. 13. Chloro- and fluoro-benzoic acids and amides. By H. G. BRAY, R. C. CLOWES, W. V. THORPE, K. WHITE and P. B. WOOD

The effect of heat on the microbiological and anti-anaemic properties of human gastric juice mixed with vitamin B_{12}. By G. H. SPRAY

The effects of glucose and potassium on the metabolism of pyruvate in *Lactobacillus arabinosus*. By P. M. NOSSAL

The *Striga* germination factor. 2. Chromatographic purification of crude concentrates. By R. BROWN, A. W. JOHNSON, E. ROBINSON and G. J. TYLER

Reduction of dehydroascorbic acid by bacteria. 1. Reducing mechanisms of *Escherichia coli*. By B. P. EDDY

Oxidation of phenylacetic acid by *Penicillium chrysogenum*. By D. J. D. HOCKENHULL, A. D. WALKER, G. D. WILKIN and F. G. WINDER

Studies in the biochemistry of micro-organisms:

85. Cyclopolic and cyclopaldic acids, metabolic products of *Penicillium cyclopium* Westling. By J. H. BIRKINSHAW, H. RAISTRICK, D. J. ROSS and C. E. STICKINGS

Appendix—Antifungal tests. By G. SMITH

86. The molecular constitution of mycophenolic acid, a metabolic product of *Penicillium brevi-compactum* Dierckx. Part 3. Further observations on the structural formula for mycophenolic acid. By J. H. BIRKINSHAW, H. RAISTRICK and D. J. ROSS

87. Dihydrogladiolic acid, a metabolic product of *Penicillium gladioli* Machacek. By H. RAISTRICK and D. J. ROSS

Gladiolic acid, a metabolic product of *Penicillium gladioli*. 1. Structure. By J. F. GROVE

The behaviour of proteins at the liquid-liquid interface. By D. F. CHEESMAN

The inhibition of chymotrysin by diethyl *p*-dinitrophenyl phosphate. By B. S. HARTLEY and B. A. KILBY

Gas-liquid partition chromatography: the separation and micro-estimation of volatile fatty acids from formic acid to dodecanoic acid. By A. T. JAMES and A. J. P. MARTIN

Quantitative studies of the avidity of naturally occurring substances for trace metals. 2. Amino-acids having three ionizing groups. By A. ALBERT

Appendix—Derivation of equations. By J. N. PHILLIPS

Photometric micro-analytical estimation of acetic acid in blood and tissues. By E. CIARANFI and A. FONNESU

Studies in detoxication. 42. Fluorobenzene. Spectrophotometric determination of the elimination of unchanged halogenobenzenes by rabbits. A comparison of the oxidation *in vivo* of fluorobenzene and of benzene. By W. M. AZOUZ, D. V. PARKE and R. T. WILLIAMS

Observations on the application of fluoro-dinitrobenzene to the quantitative analysis of proteins. By G. L. MILLS

The reaction between proteins and reducing sugars in the 'dry' state. Relative reactivity in the α- and ϵ-amino groups of insulin. By HELEN M. SCHWARTZ and C. H. LEA

A micromethod for the serial determination of organically bound halogen. By S. J. PIRT and E. B. CHAIN

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **303rd Meeting** *of the Biochemical Society was held in Guy's Hospital Medical School, London, S.E.* 1, *on Friday,* 14 *December* 1951, *when the following papers were read:*

COMMUNICATIONS

The Specificity of the Toxic Effect of D-Glucosone on Mice. By S. BAYNE. (*Department of Biochemistry, Bute Medical Buildings, St Andrews*)

Although Levene & Meyer (1915) were unable to detect any chemical change when glucosone was incubated with kidney tissue, Hynd (1927) demonstrated that parenteral administration of glucosone to mice caused symptoms resembling in many respects those of insulin hypoglycaemia. On the basis of evidence presented he suggested that glucosone was an intermediate in carbohydrate metabolism formed by the action of insulin on blood glucose; however, Dixon & Harrison (1932) were unable to detect glucosone in the blood of rabbits in hypoglycaemic convulsions following a large overdose of insulin, or after incubating fructose with liver tissue in the presence of insulin. Failure of lactosone and maltosone to produce any effects also led Hynd (1927) to suggest that the toxicity might be specific for glucosone.

This specificity has been confirmed by experiments with a series of osones prepared and characterized by improved methods. Although, for mice, the lethal dose of D-glucosone is about 2·5 mg./g. body weight, no toxic effects were produced by the following osones at various levels up to 10 mg./g.: D-arabinosone, L-arabinosone, D-xylosone, L-xylosone, D-galactosone, L-glucosone, L-gulosone, D-altro-(D-*gluco*)-heptosone (sedoheptulosone), 3-methyl-D-glucosone. It would appear from these results that the toxicity of D-glucosone is not dependent on the high chemical reactivity associated with the potential keto-aldehydic osone structure.

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The Effect of D- and L-Glucosone on Yeast Fermentation. By I. L. S. MITCHELL and S. BAYNE. (*Department of Biochemistry, Bute Medical Buildings, St Andrews*)

Although Fischer (1889) observed that D-glucosone was not fermented by yeast it seemed desirable, in view of the results quoted in the preceding communication, to investigate its effect on yeast cells actively fermenting glucose.

A suspension of bakers' yeast, with cyanide as inhibitor of respiration, was incubated with 0·025M-glucose and D-glucosone (from 0·05 to 0·20M) in the Warburg apparatus. No inhibition of glucose fermentation occurred at the lowest concentration of D-glucosone, while complete inhibition was observed at the highest concentration. Preliminary indications suggest that the inhibition is competitive.

From a mixture of L-glucose and L-mannose (Sowden & Fischer, 1947) L-glucosone was prepared and purified by a procedure identical with that employed for D-glucosone. Tested under the same conditions as D-glucosone it showed no inhibitory effect at any concentration up to 0·20M.

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Plant Thiaminases. By W. C. Evans and N. R. Jones. (*Agricultural Chemistry Department, School of Agriculture, University College of North Wales, Bangor*)

The anti-aneurin activity of bracken (*Pteris aquilina*) has been reported by us previously (Evans, Jones & Evans, 1950) to result from an enzymic fission of the vitamin into its pyrimidine and thiazole moieties. The work of Haag, Stearman, Weswig, Pierce & Schubert (1950) also indicates that the action is enzymic, but Somogyi (1949, 1950) has claimed that the inactivating agent is thermostable and passes a dialysing membrane.

Re-investigation of the problem using the thiochrome method of determination, *Neurospora crassa*, mutant 9185 assay (Harrison & Miller, 1948), and rat feeding experiments has confirmed our previous findings, i.e. over 90% of the anti-aneurin activity of aqueous bracken extracts was found to be destroyed within 15 min. boiling. Two of our extracts were tested for heat stability by Mr H. N. Ridyard at the Cereals Research Station, St Albans, and his results substantially agree with ours.

The extractability of the enzyme from bracken leaves at different pH values has been examined. It rises from pH 4 to an optimum at pH 9.

Dialysis of bracken extracts separates the enzyme into thermostable 'coenzyme' and thermolabile 'apoenzyme' fractions, both of which are required for complete enzyme action on aneurin. The coenzyme is acidic in nature, soluble in ether, *iso*-butanol and chloroform. *m*-Aminobenzoic acid and 5-aminosalicylic acid appear to be able to replace the natural coenzyme, or to activate the apoenzyme in some other way.

The thiaminase activity of horse-tail species (*Equisetem arvense* and *E. palustre*), previously ascertained by chemical methods, has been confirmed by *Neurospora crassa* Mutant 9185 assay and rat-feeding experiments. Male fern (*Aspidium filix-mas*) also contains the factor. Steaming the plants abolishes their anti-aneurin properties; after such treatment they are innocuous when included in the feed of animals.

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The Aromatic Oxidative Metabolism of Certain Benzene Ring Compounds by Soil Bacteria. By B. S. W. Smith, J. D. Jones and W. C. Evans. (*Department of Agricultural Chemistry, School of Agriculture, University College of North Wales, Bangor*)

An organism has been isolated from soil-enrichment cultures which grows in a *p*-cresol-mineral salt medium (cf. Gray & Thornton, 1928). Initial steps in the metabolic pathway of this substrate are as follows:

p-cresol → *p*-hydroxybenzyl alcohol → *p*-hydroxybenzaldehyde → *p*-hydroxybenzoic acid.

The evidence for this scheme is derived from simultaneous adaptation experiments, and the chemical isolation of *p*-hydroxybenzoic acid from a growing *p*-cresol culture. *p*-Hydroxybenzoic acid is subsequently oxidized via the pathway already reported by Evans & Smith (1951).

The course of intermediate metabolism of phenylalanine and tyrosine by the aromatic ring-splitting organisms is also being investigated, since little attention has been given to these substrates (cf. Happold, 1950). Evans (1947) had previously shown that *Vibrio* 0/1 attacked the alanine side chain of tyrosine, but at what point ring fission occurred remained obscure. Using cultures of *Pseudomonas fluorescens* A 3/9 growing on either phenylalanine or tyrosine as sole carbon source, *p*-hydroxyphenylacetic acid and *p*-hydroxybenzoic acid have been detected chromatographically in the acid ether-soluble fraction. This indicates that phenylalanine may be oxidized via tyrosine to *p*-hydroxybenzoic acid prior to ring cleavage.

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Proteolysis in Brain Tissue. By G. B. Ansell, R. B. Williams and D. Richter. (*Neuropsychiatric Research Centre, Whitchurch Hospital, Cardiff*)

Brain tissue is known (Kies & Schwimmer, 1942) to contain an enzyme of the catheptic type active in the region pH 3·5–4·0. The fresh tissue also contains a system that liberates amino-acids at neutral reaction, with an optimal activity at pH 7·4. When fresh minced brain tissue suspended in phosphate-Ringer solution is incubated anaerobically at 37° the rate of formation of free amino N is of the order of 2 μg. amino N/mg. dry tissue/hr. during the first 20 min., but the activity of the system rapidly falls off and the release of amino N ceases after about 1·5–2 hr. The amino-acid liberation was followed by the ninhydrin colorimetric method (Moore & Stein, 1948) and by paper chromatography, which showed that a number of different amino-acids are liberated. It was also shown that there was no significant decrease in free peptide N during the reaction. This gave evidence that the system is not a simple peptidase.

The system is present in rat, rabbit and pigeon brain, and present in both grey and white matter. It was not activated by the addition to the minced tissue of 0·01 M-cysteine or 0·01 M-HCN; but it was strongly inhibited by 0·01 M-sodium iodoacetate. The system appears to contain more than one component, and it probably includes a peptidase and polypeptidase as well as a neutral proteinase.

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Isotope and Respiration Studies on *in vitro* Fatty Acid Synthesis by Mammary Tissue: Species Differences in Effects of Insulin and Glycerol. By Judith H. Balmain, S. J. Folley and R. F. Glascock. (*National Institute for Research in Dairying, University of Reading*)

The following conclusions were drawn from respiration and acid-change measurements on lactating mammary gland slices. (1) They effect net fatty acid synthesis *in vitro*, ruminant tissue being effective in acetate alone but more so in acetate + glucose, while non-ruminant tissue utilizes acetate for fat synthesis only in presence of glucose (Folley & French, 1950). (2) Insulin *in vitro* stimulates fatty acid synthesis by rat mammary tissue in glucose alone or glucose + acetate but not in acetate alone; sheep tissue, however, is inert to insulin under all conditions tried (Balmain & Folley, 1951). (3) Glycerol has a qualitatively similar effect to insulin on rat tissue in glucose + acetate, and with sheep tissue stimulates fat synthesis in acetate alone, but not in glucose + acetate (Balmain & Folley, 1951); it is thus a limiting factor in fat synthesis under these conditions.

Since respiratory data cannot provide conclusive evidence on this question it has now been studied by means of labelled acetate, 2 or 3 g. batches of lactating mammary slices being incubated (37°) with $CH_3{}^{14}COONa$, with and without glucose, in bicarbonate-CO_2 buffer. Total fatty acids were isolated from the slices after 3 hr. and the calcium salts counted at infinite thickness.

With rat tissue (six experiments) in acetate, the specific activities of the calcium salts hardly differed from those from control slices killed at zero time; insulin did not increase the count and glycerol did so only very slightly. In glucose + acetate, however, the incorporation of acetate was considerable and in the presence of insulin (1 i.u./ml.) or glycerol (0·2%), the specific activities were further increased by about twice. In this species, glycerol clearly did not replace glucose as a stimulant of fatty acid synthesis from acetate.

On the other hand, sheep mammary slices (six experiments) in acetate alone incorporated considerable quantities of acetate into fatty acids and still more (about three times as much) in acetate + glucose. Insulin had no effect either in acetate alone or in acetate + glucose, but glycerol almost doubled the incorporation from acetate as sole substrate, but had no effect in acetate + glucose. The deductions from respiratory and acid-change data have thus been confirmed in every particular.

Glycerol was isolated from the glyceride fractions as the tri-*p*-nitrobenzoate and all samples were virtually non-radioactive; the slices therefore probably do not use acetate for glycerol synthesis. The origin of the glycerol used for glyceride formation by sheep slices metabolizing acetate alone is therefore of interest.

We are indebted to Dr K. Hallas Møller of the Novo Terapeutisk Laboratorium, Copenhagen, for generous supplies of insulin free from glycogenolytic factor.

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The Regeneration of Red Cell and Plasma Cholinesterases in Humans and Dogs following Poisoning by bis(*iso*propylamino)fluorophosphine oxide. By S. CALLAWAY, D. R. DAVIES and J. E. RISLEY. (*Ministry of Supply, Porton, Wilts*)

Recently three people, two men and one woman, were poisoned by a new organo-phosphorus insecticide, bis(*iso*propylamino)fluorophosphine oxide (A), $[(CH_3)_2CHNH]_2PFO$.

Thompson (1951) has shown that this compound is a potent inhibitor of cholinesterase *in vitro*. It is also an inhibitor of this enzyme *in vivo*, since all three subjects exhibited typical cholinergic symptoms after working with (A) intensively for 3 or 4 days. Immediately on developing symptoms they were treated with atropine, and samples of their blood were sent to us for analysis and the cholinesterase values were determined.

Approximately a week later, two patients exhibited paralysis of the legs and hands, a condition which persisted for several weeks. In view of the rarity of clinical material of this kind we have followed the rate of return of the red cell and plasma cholinesterase.

The red cell enzyme returns in linear fashion at approximately 1% per day. This is in agreement with the finding of Grob, Lilienthal, Harvey & Jones (1947) following the administration of di-*iso*propyl fluorophosphonate (DFP) to man. The plasma enzyme returns exponentially, but very much more slowly than following the destruction of this enzyme by DFP or tetraethyl pyrophosphate (TEPP) (Grob *et al.* 1947). For example, in 10 days 60% of the plasma enzyme had reappeared after DFP poisoning, whilst in the case of the present compound only 25% had returned. It took approximately 40 days for complete return after DFP, whereas only 60% of the plasma enzyme had returned after compound (A).

DFP is an irreversible inhibitor (Aldridge, 1950), and it is generally believed that the combination of enzyme and inhibitor results in the destruction of the enzyme. The reappearance of new enzyme is therefore taken to indicate the formation of new protein (Mazur & Bodansky, 1946; Koelle & Gilman, 1946). Another property of DFP which is pertinent to the present issue is its extreme specificity. It is a highly specific inhibitor of cholinesterase, and there is no evidence that it in any way interferes with the processes affecting the production of new enzyme.

Therefore, if compound (A) behaved similarly, the rate of regeneration of both the red cell and plasma enzymes should be comparable with that following DFP. Since this is not so, compound (A) probably possesses a different mode of action.

Experiments have also been carried out on dogs to compare the action of compound (A), tri-*ortho*-cresyl phosphate (which Thompson (1951) suggests may also have a similar action) and DFP. The results were discussed.

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Citrulline as an Intermediate in the Breakdown of Arginine by *Streptococcus faecalis*. By V. A. KNIVETT (introduced by E. F. GALE). (*Medical Research Council Unit for Chemical Microbiology, Biochemical Laboratory, University of Cambridge*)

Hills (1940), in a study of ammonia production by Gram-positive cocci, found that *Streptococcus faecalis* attacks arginine with the liberation of ornithine, carbon dioxide and ammonia. Since urea was not an intermediate in the breakdown, he postulated the action of a new enzyme called 'arginine dihydrolase'.

When washed suspensions of *Strep. faecalis* are incubated with arginine, complete disappearance of the arginine takes place, but only 70–80% can be accounted for by the known products of the reaction. Attempts have been made to study the reaction using cell-free preparations or cells treated with the detergent cetyltrimethylammonium bromide (CTAB) or with acetone; in these cases preparations have been obtained which attack arginine with the liberation of ammonia, 1 mol./mol. arginine broken down, but no carbon dioxide or ornithine. Chromatographic examination of the products reveals the presence of a new ninhydrin-positive material which has been identified as citrulline. The formation of citrulline from arginine by CTAB-treated cells is very rapid ($Q_N = \mu$l. NH_3-N liberated from arginine/hr./mg. dry weight

of cells = 750 at 37° and pH 5·6), and the reaction proceeds to completion. The reaction is inhibited by certain long-chain amidines, diguanidines and substituted guanidines, including paludrine.

Citrulline has been identified amongst the products of the breakdown of arginine by intact cell suspensions; it is now possible to account for all the arginine disappearing. Citrulline is attacked only very slowly by intact cells and not at all by CTAB-treated or acetone-dried cells in the absence of adenosinetriphosphate (ATP). In the presence of sufficient ATP, CTAB-treated cells metabolize citrulline with the production of ornithine, ammonia and carbon dioxide.

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Long-chain Unsaturated Fatty Acids as Essential Bacterial Growth Factors. Further Studies with *Corynebacterium* 'Q'. By B. W. Boughton and M. R. Pollock. (*Department of Chemistry, Birkbeck College, University of London, and National Institute for Medical Research, Mill Hill, London, N.W.* 7)

It has been previously reported (Pollock, Howard & Boughton, 1949) that an oleic acid-requiring *Corynebacterium* species ('Q') could grow on a number of related unsaturated fatty acids from natural sources (elaidic, linoleic, linolenic, petroselinic and palmitoleic acids) as well as on oleic acid itself, although the saturated acids tested (lauric, myristic, palmitic and stearic acids) were quite ineffective.

This work has now been extended to include a number of synthetic compounds, less closely related to oleic acid, using the same technique for testing activity as previously.

After showing that synthetic oleic, elaidic and *cis*-palmitoleic acids gave the same quantitative results as purified preparations from natural sources, the following new compounds were tested, with results summarized below.

Positive (growth approximately equivalent to oleic acid): *cis*- and *trans*-vaccenic acids, *cis*- and *trans*-heptadec-9-enoic acids, *trans*-palmitoleic acid.

Negative (no growth): *cis*- and *trans*-octadec-4-enoic acids, *cis*- and *trans*-gadoleic acids, *cis*- and *trans*-myristoleic acids, *cis*-undec-9-enoic acid, *cis*-undec-10-enoic acid.

Apart from *trans*-myristoleic acid and *cis*-octadec-4-enoic acid, which gave very slight growth in the higher concentrations, the differences were clear-cut.

A further number of saturated acids—acetic, azelaic, pelargonic, pentadecanoic and heptadecanoic acids—were also tested and gave negative results. Two mono-methyl substituted oleic acids (CH_3 group in the 9 and 12 positions respectively) supported growth, though this was not estimated quantitatively; while a dimethyl- and a tetramethyl analogue (7:11-dimethyloleic acid and 5:7:13:17-tetramethyl oleic acid respectively) were both ineffective.

The following points are worth noting. (1) The heptadec-9-enoic acids, with an odd number of C atoms, were as effective as oleic acid. (2) Fatty acids with the *trans*-configuration were as effective as their corresponding *cis*-isomers. (3) While the hydrocarbon 'tail' of oleic acid can be shortened by at least two C atoms without affecting its growth-promoting properties, the addition of two C atoms completely abolishes activity. (4) The double bond can be shifted away from the 9:10 position of oleic acid at least to 11:12 and to 6:7 (but not as far away as 4:5) without marked loss of activity.

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The Prevention of Colour-Fading in the Folin and Wu Estimation of the Blood Sugar. By H. Lehmann and E. Silk. (*Department of Pathology, St Bartholomew's Hospital, London, E.C.* 1)

The colorimetric blood-sugar estimation of Folin & Wu (see Harrison, 1947) suffers from the disadvantage that the colours are not stable. The instability remains even if precautions as to purity of chemicals are taken as described by King & Garner, (1947).

Although the absolute loss of colour tends to be greater the deeper the colour, the relationship is not proportional, and it is not possible to introduce a correction. Fading is particularly inconvenient when low blood-sugar values are measured, and a result of 60 mg./100 ml. can give readings corresponding

to 35–40 mg./100 ml. after 20 min. delay. The phenomenon is largely restricted to colours of a wavelength of 550 mμ. and above. Thus, it is much more obvious with photoelectric colour determination where a red filter is used than with a visual colorimeter when the whole of the spectrum is measured.

The loss of colour is greater the higher the temperature and depends on the amount of water added to make up the final volume.

Readings not subject to fading can be obtained by:

(1) Cooling the Folin-Wu tubes in ice water immediately after they have been filled to mark and leaving them there until the measurement of colour is made.

(2) Using a blue or green filter which excludes the red part of the spectrum in which fading occurs.

(3) Not diluting with water after the phosphomolybdate reagent has been added.

All three methods have disadvantages. Working with ice water is unsuitable for routine estimations. By not registering the red part of the spectrum one excludes the major changes in light extinction. If there is no dilution after adding the phosphomolybdate reagent, the readings become too high and the volume is too small for some types of colorimeter.

The fading on adding water was found to be due to the lowering of the phosphoric acid concentration. It does not take place if the tubes are filled to the mark with a 11·55% (w/v) solution of phosphoric acid. Dilutions to 12·5, 25 or 50 ml. will retain their colour at room temperature for more than 8 hr.

In practice when 12·5 ml. are the usual final volume one adds to the mixture of 2 ml. substrate and 2 ml. copper tartrate reagent after 6 min. boiling and 1 min. cooling, 3 ml. of the following modified phosphomolybdate reagent: phosphomolybdate reagent of Folin & Wu, 500 ml.; phosphoric acid (89%, w/v), 215 ml.; water, 35 ml.

After adding this reagent one fills up to the mark with water. A final volume of 12·5 ml. will be stable at room temperature. Even on dilution with water to 25 ml. the fading will be below 5% in 2 hr.

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Blood Levels of 5-Nucleotidase (Adenosine-5-phosphatase). By T. F. Dixon. (*The Institute of Orthopaedics, Royal National Orthopaedic Hospital, London, W.* 1)

In view of the high adenosine-5-phosphatase content of ossifying cartilage and the likelihood that this enzyme is concerned with co-calcification processes it was thought of interest to determine the blood levels in a variety of disease states. This led to the unexpected result that in bone diseases, such as Paget's disease and rickets, although the non-specific 'alkaline phosphatase' was, as is well known, raised, yet the adenosine-5-phosphatase was normal. In jaundice, either infective or obstructive, the blood levels of both enzymes were raised. Certain tubercular spine cases had abnormal adenosine phosphatase levels, and attention was thus directed towards liver lesions in these conditions. Various theories were put forward to explain these findings.

The Cholinesterases of the Human Central Nervous System. By Margery G. Ord and R. H. S. Thompson. (*Department of Chemical Pathology, Guy's Hospital Medical School, London*)

Although the levels of acetylcholine hydrolysis by different parts of the human brain have been studied in some detail, the distribution of pseudo-cholinesterase in this tissue has not been described. Contrary to the earlier view, however, benzoylcholine is now known to be hydrolysed by the brains of a number of different animal species (Augustinsson, 1948; Zeller, 1949; Ord & Thompson, 1950; Burgen & Chipman, 1951). We have therefore compared the relative rates of hydrolysis of acetylcholine, acetyl-β-methylcholine (MCh), benzoylcholine (BCh), butyrylcholine (BuCh) and tributyrin by preparations from different areas of human brain.

In all the areas studied a measurable degree of hydrolysis of BCh and BuCh was observed. This BCh-hydrolysing enzyme is distinct both from the

true cholinesterase and from the tributyrinase activity of these brain extracts. It appears to be a 'butyrocholinesterase' (Sturge & Whittaker, 1950), closely resembling the pseudo-cholinesterase present in human plasma. The white fibre tracts of human cerebrum contain significantly more of this pseudo-cholinesterase than the grey matter. The rate of BCh hydrolysis by white matter was in every experiment greater than the rate of hydrolysis of MCh by this area. It is suggested that this enzyme may be connected with processes occurring in the myelin sheaths or the connective tissue of the nervous system rather than directly with the properties of the axis cylinders.

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Fructosans of Barley. By H. K. Porter and J. Edelman. (*Research Institute of Plant Physiology, Imperial College, London, S.W.* 7)

Analysis of fractions containing combined fructose isolated during preparation of a sample of fructosan from barley leaves (Archbold & Barter 1935) indicated that material consisting mainly of 2:6-linked fructofuranoside units (Haworth, Hirst & Lyne, 1937) and described as having $[\alpha]_D^{15°}$ −37·8°, and yielding on hydrolysis 94 % fructose and 6 % aldose, might be the least soluble fraction of a series of fructose polymers. In the more soluble fractions the proportion of aldose varied from 5 to 10 %, $[\alpha]_D^{15°}$ from −37° to −25°, and the degree of hydrolysis by invertase under standard conditions varied from 4 to 30 %. Moreover, unfractionated stem extracts when subjected to invertase action showed an initial rate of fructose production higher than that expected from the compound with $[\alpha]_D^{15°}$ −37·8°, while simultaneous aldose production amounted to 18 % of the fructose. Slow fructose production continued until hydrolysis was complete, while only traces of aldose appeared in the later stages. Finally, during elongation of the stem, combined aldose is formed simultaneously with combined fructose. The amount averaged 14 % of the fructose, with a tendency for the proportion to fall as fructosans reached peak values.

Application of the chromatographic technique used by Bacon & Edelman (1951) for investigating artichoke extracts confirmed that extracts of barley leaves and stems contain a series of saccharides. At least four spots other than those due to glucose, fructose and sucrose, separated discretely on the paper chromatograms developed in butanol-acetic acid, and material with yet lower R_F extended to the starting line. The relative amounts of these components varied as between leaf and stem, and with the stage of plant development, but the two spots with R_F immediately lower than that of sucrose were usually present in only small amounts. All the spots contained both fructose and glucose, thus identifying the aldose as glucose, and treatment with invertase on the paper, using two-dimensional chromatography, showed that these were the only products of hydrolysis and that rate of inversion fell with decreasing R_F. Lastly, the colour of the spots indicated that the proportion of glucose in the several compounds decreases with R_F.

Combined fructose in barley is therefore distributed among compounds ranging from sucrose and oligosaccharides rather easily split by invertase, to longer chain polymers somewhat resistant to invertase action. The lower polymers and some, if not all, of the higher ones contain glucose.

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Amino-acid Composition of Basic Nuclear Proteins. By E. J. EADIE and G. LEAF. (*Biochemistry Department, University of Glasgow*)

The amino-acid compositions of two preparations of 'histone' obtained respectively from isolated rat liver nuclei and from calf thymus have been determined. The chromatographic methods of Stein & Moore (1948) and Moore & Stein (1949) were used, and the results, in terms of amino acid N as percentage of total N, were as follows.

Rat liver 'histone': alanine, 10·7; NH_3, 7·2; arginine, 23·2; aspartic acid, 4·45; glutamic acid, 5·68; glycine, 5·94; histidine, 1·62; leucine and isoleucine, 10·0; lysine, 11·6; phenylalanine, 1·65; proline, 2·1; serine, 5·0; threonine, 5·6; tyrosine, 1·07; valine, 3·9.

Calf thymus 'histone': alanine, 8·5; NH_3, 9·5; arginine, 20·1; aspartic acid, 5·4; glutamic acid, 6·9; glycine, 6·2; histidine, 4·2; isoleucine, 3·7; leucine, 6·1; lysine, 8·5; phenylalanine, 1·17; proline, 3·0; serine, 4·7; threonine, 5·3; tyrosine, 0·5; valine, 6·1.

Similar analyses have been recently reported for calf thymus by Hamer (1951) and Daly, Mirsk & Ris (1951), and for rat liver histone by Brunish, Fairley & Luck (1951). All the preparations show common features; they contain only small amounts of the aromatic amino-acids phenylalanine and tyrosine and relatively large amounts of the basic amino-acids arginine and lysine. Cystine and methionine are absent or present in very small amounts. In the case of rat liver 'histone' our results are not very different from those of Brunish *et al.* With calf thymus histone preparations, however, the results from different laboratories show discrepancies which are beyond the limits of experimental error. This confirms the suggestions of Stedman & Stedman (1950) and Daly *et al.* (1951) that histone preparations are mixtures of proteins with similar properties. The assumption that any histone preparations consist of a single homogeneous protein would require rigorous proof.

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The Determination of Volatile Fatty Acids in Blood. By R. SCARISBRICK. (*Department of Animal Pathology, University of Cambridge*)

The steam-distillation method for the determination of small quantities of volatile fatty acids elaborated by McClendon (1944) and subsequent modifications (Kiddle, Marshall & Phillipson, 1951) are time consuming and of limited accuracy. Fundamental difficulties are associated with the removal of volatile acids quantitatively from solution by steam distillation and the titration of the resulting very weakly acidic distillates. Investigation and consideration of these lead to the development of the following method which offers certain advantages, particularly in speed and, because the distillation is performed at pH 4, in specificity.

Liquid paraffin (1 ml.) containing 2% of sec-octyl alcohol is put in the inner chamber of a Markham apparatus and followed by 1 ml. blood, 0·5 ml. 10% potassium oxalate and 0·5 ml. 5% oxalic acid. Distillation in steam is commenced, taking care that the mixture is heated first from within by the steam. Two 40 ml. volumes of distillate are collected, the second constituting a blank. To the distillate is added 1 ml. of 0·01% bromthymol blue prepared by diluting a stock 0·2% solution which has been neutralized to pH 7·0. The distillates are aerated for 10 min. with acid and alkali-free air and then titrated with 0·01 N-sodium hydroxide to pH 7·0.

A film of protein and paraffin remains adherent to the glass of the Markham apparatus: it is unnecessary to clean this off on each occasion, but when it has reached undesirable proportions it can be removed with hot 0·5% sodium hydroxide.

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A Study of the Phosphate Esters in Muscle by means of Paper Chromatography. By P. C. CALDWELL. (*Biophysics Research Unit, University College, London*)

The methods described by Hanes & Isherwood (1949) for the separation of organic phosphates on a paper chromatogram and their subsequent quantitative estimation have been adapted to the study of these compounds in muscle. A two-dimensional chromatogram in which the solvents *n*-propanol/ammonia/water and *tert.*-butanol/picric acid/water are used successively splits the esters into five fractions, each of which can be determined independently. These are, adenosinetriphosphate (ATP), adenosinediphosphate (ADP), orthophosphate, hexosediphosphate and creatine phosphate + hexosemonophosphates + adenylic acid. If the sum orthophosphate + creatine phosphate is determined by the usual methods, individual values for creatine phosphate and hexosemonophosphates + adenylic acid can be obtained.

The analyses which have been carried out so far on frog muscles subjected to varying conditions are in good agreement with those carried out by others (e.g. Eggleton & Eggleton, 1929; Lundsgaard, 1930; Causey & Harris, 1951) by means of the more conventional methods. These muscles are shown to contain about three times as much of the various esters as the much slower tortoise and snail muscles.

The values obtained for ADP suggest that this accounts for 5–15 % of the pyrophosphate fraction of intact muscle. No clear-cut indication of a formation of this substance from ATP when a muscle is stimulated has as yet been obtained.

Very little, if any, hexosediphosphate can be detected in intact, unpoisoned muscles, and the relative concentrations suggest that this substance may be in equilibrium with orthophosphate and hexosemonophosphate. The phosphohexokinase reaction is very far from equilibrium (cf. Ogston & Smithies, 1948), and experiments with muscles poisoned with iodoacetate indicate that it may be controlled by the same mechanism as the contractile process. The pyrophosphate which is left in these poisoned muscles when they have been stimulated to exhaustion is shown to be still predominantly in the form of ATP.

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Bile Salts of the European Frog, *Rana temporaria* and of *Alligator mississippiensis*. By G. A. D. HASLEWOOD. (*Guy's Hospital Medical School, London, S.E.* 1)

The chief bile salt of *Rana temporaria* is apparently the sulphate ester of an alcohol, 'ranol' $C_{27-28}H_{43-45}(OH)_5$. A tetra-acetate of this was easily isolated after acid hydrolysis of the sulphate. Alkaline hydrolysis, on the other hand, gave a product which yielded crystalline derivatives apparently of an 'anhydroranol', differing from ranol by H_2O. These derivatives may contain an oxide ring, formed during elimination of the sulphate with a suitably placed OH group. The formation of such substances may explain some of the confusion in the literature about the nature of neutral substances from amphibian biles. The molecule of ranol may contain $—CH_2OH$ and secondary hydroxyl groups at C_3, C_7 and C_{12} in the steroid nucleus.

Bile of *Alligator mississippiensis* (obtained through the good offices of Dr W. H. Pearlman of the Jefferson Medical College of Philadelphia) after hydrolysis yielded a mixture of acids, one of which (of m.p. approx. 170°) probably has the formula $C_{27}H_{46}O_5$ and has been characterized as the ethyl ester, m.p. 136–138°, and dehydro-derivatives. On the hypotheses put forward by Haslewood & Wootton (1950), these findings suggest that both the creatures examined are evolutionarily of a primitive type. These and similar results may be of special importance in a study of a possible relationship between the chemical nature of bile salts and theories of evolution.

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Anomalies in Partition Chromatography: The Nature of 'Vitamin B_{12d}'. By E. LESTER SMITH. (*Glaxo Laboratories Ltd., Greenford, Middlesex*)

Our claim that 'vitamin B_{12d}', the product arising on removal of nitrite from vitamin B_{12c} (nitrito-cobalamin) (Lester Smith *et al.* 1951), differed from the expected product, vitamin B_{12b}, rested mainly upon their apparent separation by partition chromatography. The spurious character of this separation was demonstrated in various ways. Pure vitamin B_{12b} often gave two (and sometimes three) slow-moving zones on kieselguhr columns run with wet *n*-butanol. Their proportions were not constant and each divided again on transfer to new columns. Addition of 15% of phenol to the solvent to give a somewhat higher *R* value eliminated this multiple zoning, even with 'mixtures' of vitamins 'B_{12d}' and B_{12b}. When each in turn was made radioactive and chromatographed with the other, the specific activity was constant from top to bottom of the red band—a rigorous proof of identity.

On butanol columns double zoning with a pure solute of low *R* value can result from the top of the column being either too wet or too dry. A wet film can trap part of the solute, while what escapes travels at the normal rate; this was demonstrated with a green dye as well as with vitamin B_{12b}. The latter is adsorbed slightly by dry kieselguhr from solutions in butanol, so that a dry top section can also trap parts of this solute; the adsorbed material gathers into a band on subsequent development with fully saturated butanol. Exceptionally a succession of both these faults gives rise to three zones. These discontinuities in the columns arise from the use of solvent either containing a trace of excess water in fine suspension or not quite fully saturated. In turn these conditions may be due to over-hasty preparation of 'water-saturated' solvent (prolonged shaking is needed, and excess water settles out very slowly) or to changes of a few degrees in laboratory temperature.

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Structure and Stability of Some Cobalamins. By E. LESTER SMITH. (*Glaxo Laboratories Ltd., Greenford, Middlesex*)

The B_{12} vitamins may be represented by the partial formula (I) (Buhs, Newstead & Trenner, 1951).

$$\begin{bmatrix} - & - & X \\ Co^{+++} & & \\ 0 & 0 & 0 \end{bmatrix} \qquad \begin{bmatrix} - & - & H_2O \\ Co^{+++} & & \\ 0 & 0 & 0 \end{bmatrix}^{+}$$

(I) (II)

$$\begin{bmatrix} - & - & CN^- CN^- \\ Co^{+++} & & \\ 0 & 0 & \end{bmatrix}^{-} \qquad \begin{bmatrix} - & - & SO_3^{--} \\ Co^{++-} & & \\ 0 & 0 & \end{bmatrix}^{-}$$

(III) (IV)

When *X* is a negative ion with strong co-ordinating tendencies the valencies are internally satisfied and a stable neutral cobalamin results, e.g. vitamin B_{12} (cyano-cobalamin) and vitamin B_{12c} (nitrito-cobalamin) (Lester Smith *et al.* 1951), where *X* is CN^- or ONO^- respectively. Replacement by the neutral molecule H_2O yields the basic aquo-cobalamin (vitamin B_{12b}, II) (Cooley *et al.* 1951). Its ionized compounds with mineral acids are best regarded as salts rather than as 'chloro-cobalamin', etc. The term 'iodo-cobalamin' is then available for the loose adduct with elemental iodine. Another series of cobalamins is typified by the purple adduct with excess cyanide, dicyano-cobalamin (III). The two anions give the co-ordination complex a nett negative charge; they presumably displace not only *X* but one of the neutral O groups, and spectroscopic evidence suggests that this is the linkage between cobalt and the $N_{(3)}$ atom of the benziminazole group (Cooley *et al.* 1951). Instead of two cyanide groups one can have, for example, two thiocyanate groups. Also mixed acidic adducts such as cyano-thiocyanato-cobalamin can be prepared. Or a bivalent anion can be present, as in the sulphite adduct (IV).

Adducts of this class are characterized by instability; in solution they exist only in presence of an excess of the appropriate ion, and it has not proved possible to prepare pure specimens. Their formation and their acidic character can be demonstrated by changes in absorption spectra and partition coefficients. They extract into solvents more readily at acid pH and less readily at alkaline pH than the corresponding neutral cobalamins.

Vitamin B_{12} containing radioactive carbon in the cyanide group exchanges this with ordinary CN^- very rapidly in alkaline solutions, but only slowly at acid pH. Tracer studies reveal little replacement of cyanide by $CN^{35}S^-$ even at alkaline pH, and even less by nitrite.

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The Fate of 4:6-Dinitro-*o*-cresol (DNOC) in the Rabbit. By J. N. Smith, R. H. Smithies and R. T. Williams. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W.* 2)

The weed-killer and insecticide DNOC is highly toxic (Bidstrup & Payne, 1951) and acts as a cumulative poison in man (Harvey, Bidstrup & Bonnell, 1951). Little is known about its metabolism, except that is slowly excreted and only 1–2% is excreted unchanged (Harvey *et al.* 1951).

Rabbits tolerated oral doses of 20–30 mg./kg., but more than 30 mg./kg. was invariably lethal. Urine from rabbits which had received non-lethal doses of DNOC was collected for 48 hr. and extracted with ether before and after boiling with 2 N-HCl. Less than 20% of the dose was recovered as metabolites in these extracts.

The metabolites of DNOC were separated by paper chromatography and identified by colour reactions and spectroscopy. The main compounds found were unchanged DNOC, conjugated DNOC, 6-acetamido- and 6-amino-4-nitro-*o*-cresol, roughly three times as much of the reduced compounds being excreted as DNOC. In addition, substances with the same chromatographic behaviour as 4-amino-6-nitro-*o*-cresol and 3-amino-5-nitrosalicylic acid were present in small amounts.

6-Acetamido- and 6-amino-4-nitro-*o*-cresol were not toxic to rabbits in doses of 500 mg./kg., suggesting that reduction of one of the nitro groups of DNOC detoxicates it. The 6-amino compound is partly oxidized in the rabbit to 3-amino-5-nitro-salicylic acid and also partly acetylated. 6-Acetamido-4-nitro-*o*-cresol is partly excreted by rabbits as 6-acetamido-4-nitro-*o*-cresylglucuronide. Sodium salt, decomp. 160–170°; $[\alpha]_D^{20°} - 29{\cdot}8°$ in water.

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The Excretion of 1-Naphthol and of 1:2-Dihydroxy-1:2-dihydronaphthalene by Rats Injected with Naphthalene. By E. Boyland and G. H. Wiltshire. (*Chester Beatty Research Institute, The Royal Cancer Hospital, London, S.W.* 3)

Rats and rabbits dosed with naphthalene excrete 1-naphthol in free and conjugated forms and also 1:2-dihydroxy-1:2-dihydronaphthalene ('naphthalene diol'). Rats and rabbits injected with 1-naphthol do not appear to excrete the naphthalene diol nor do rats dosed with naphthalene diol excrete 1-naphthol. After the first injection of naphthalene into rats or rabbits the urine contains relatively much more 1-naphthol and 1-naphthylsulphate than naphthalene diol. With repeated daily injections of naphthalene the relative and absolute amounts of naphthalene diol excreted increase, and at the same time rats become resistant to the action of naphthalene. The results indicate that the formation of the naphthalene diol is an adaptive process which increases with training. The adaptation is possibly analogous to the increased ability of rat liver to metabolize tryptophan (Knox & Mehler, 1950) and to some other examples of drug resistance. Experiments with liver slices incubated with naphthalene showed no evidence of 1-naphthol formation, and the rate of naphthalene diol formation appeared to be no greater in the liver tissue of trained rats than that of fresh rats.

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The Effect of Vitamin B_{12} on the Biosynthesis of Choline Methyl Groups in the Rat. By H. R. V. Arnstein and A. Neuberger. (*The National Institute for Medical Research, Mill Hill, London, N.W. 7*)

In an earlier communication (Arnstein & Neuberger, 1951) we showed that vitamin B_{12} increased the conversion of the α-carbon atom of glycine to choline methyl groups in the rat. However, even when vitamin B_{12} was given, less than one methyl group/choline molecule was derived from this precursor, which might indicate that the α-carbon atom of glycine is not a *direct* precursor. Since formate and the β-carbon atom of L-serine are also methyl group precursors (Arnstein, 1951), the biosynthesis of choline methyl groups from these sources has been compared.

The basal diet contained all the vitamins except vitamin B_{12} and the essential amino-acids, including L-cysteine (0·7%) with the exception of methionine which was replaced by DL-homocystine (1%). Choline (5 mg./rat) and/or vitamin B_{12} (2·5 μg./rat) were added daily. [α-^{14}C.] Glycine (0·5%; 0·743 μc./mmol.), [β-^{14}C] L-serine (0·7%; 0·219 μc./mmol.) or sodium [^{14}C] formate (0·1%; 2·81 μc./mmol.) were fed for 19 days. At the end of the experiment the animals were killed and the choline was isolated as the chloroplatinate and degraded to trimethylamine chloroplatinate (du Vigneaud, Cohn, Chandler, Schenk & Simmonds, 1941).

The distribution of ^{14}C in the choline indicated that vitamin B_{12} has little or no effect on the conversion of formate or the β-carbon atom of serine to choline methyl groups. The earlier finding of an increased utilization of the α-carbon atom of glycine was confirmed, but incorporation of isotope into both the ethanolamine moiety and methyl groups of choline was much lower when compared with [β-^{14}C] serine or formate. This appears to indicate that the α-carbon atom of glycine is not converted directly to a one-carbon precursor of methyl groups, but is first incorporated into the β-carbon atom of serine (Sakami, 1949). Since vitamin B_{12} seems to have no effect on the formation of choline from serine it may be concerned with the formation of serine from glycine. Such a mechanism would also explain the observation that vitamin B_{12} is able to counteract glycine toxicity in the chick (Menge & Combs, 1950).

Since vitamin B_{12} lowers the requirement for choline it appears probable that it is concerned with the formation of methyl groups from precursors not yet investigated.

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Polynucleotides and End Groups in Ribonucleic Acids. By R. Markham and J. D. Smith. (*Agricultural Research Council Plant Virus Research Unit, Molteno Institute, University of Cambridge*)

Investigating the enzymic breakdown of ribonucleic acids we have isolated ten dinucleotides, four trinucleotides and four dinucleoside monophosphates and larger fragments. All the dinucleotides isolated are linked through the ribose residues at carbon atoms 3′ (or 2′) and 5′. From fragments obtained by ribonuclease action, which breaks some 5′ internucleotide ester links, it appears that the factor deciding whether a link in a nucleotide chain will be broken by the enzyme is that a pyrimidine nucleoside 2′:3′ monohydrogen phosphate should be formed first. The enzyme then breaks this down to an ordinary nucleotide. For example, uridine 5′-(uridine-3′ (or 2′)-phosphate)-ester, 2′:3′ monohydrogen phosphate is converted by ribonuclease first to uridine 2′:3′ monohydrogen phosphate and then to uridylic acid.

Because of this specificity there are in ribonuclease digests a number of polynucleotides, terminated at one end by single pyrimidine nucleotide residues joined by their 5′ carbon atoms, which are not attacked by ribonuclease. These range from forms like uridine 5′-(guanosine-3′ (or 2′)-phosphate)-ester, 3′ (or 2′)-phosphate to the 'ribonuclease-resistant core', which is probably a mixture of polynucleotides of four to five residues in length. All these polynucleotides may be dephosphorylated by phosphomonoesterase and the product isolated.

On alkaline hydrolysis these derivatives yield purine nucleotides plus a pyrimidine nucleoside. For example, guanine-uracil dinucleotide treated with monoesterase goes to a substance which on alkaline hydrolysis yields equivalent quantities of guanylic acid and uridine.

A small amount of free adenosine and guanosine, 2′:3′ monohydrogen phosphates is liberated from carefully prepared nucleic acids by ribonuclease. These must be terminal groups of chains, and in yeast nucleic acid comprise about 1 in 66 of the residues. By treatment of whole ribonucleic acids by pure phosphomonoesterase other end groups are revealed consisting of pyrimidine nucleotide residues. It seems probable, therefore, that ribonucleic acids are mixtures. Commercial yeast nucleic acid has shorter chains containing such end groups as purine nucleoside 2′ and 3′ phosphates (Markham & Smith, 1951), which are probably artifacts.

It appears that ribonuclease is a specific pyrimidine nucleotide ester diesterase.

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Organic Constituents of Enamel. By M. V. Stack. (*Department of Dental Medicine, Guy's Hospital, London*)

A wet-ashing procedure (Johnson, 1949) was applied to human enamel purified by repeated stirring with bromoform, as suggested by Manly & Hodge (1939). Less than 3% loss occurred in reducing the dentine impurity to 0·2% by this procedure. The organic content of partially purified enamel compared closely with a microscopical assessment of the degree of contamination with dentine (Stack, 1951). Among 10 batches of enamel, totalling 115 g., the standard deviation of organic content was 5% of the mean, as expected from previous analyses of 50 enamel samples from individual teeth, when a normal distribution was observed (Stack & Williams, 1951). Smaller batches of mechanically separated premolar enamel of similar purity showed a much greater variation, judging by nitrogen analyses (Anderson, 1949).

Aqueous extracts of purified enamel contained 7–9% of the total organic matter. On dialysis of washed enamel against 0·1 M-H_3PO_4, soluble and insoluble enamel proteins were retained in yields of 0·1 and 0·2% respectively, the recovery of organic matter being incomplete. Losee & Hess (1949) isolated 0·3–0·5% insoluble protein by demineralizing enamel with 2% acetic acid, but failed to detect soluble protein in their dialysates. Similar ratios of nitrogen to organic matter (Stack, 1950) were observed in extracts, dialysates and whole enamel, but the ratio was greater in the case of the insoluble protein (eukeratin, N content 12·5–13·5%, 3–5% ash). Assuming that material not accounted for has a similar composition, it is concluded that most enamel samples contain 0·4–0·8% organic matter including 0·1% citric acid (Zipkin & Piez, 1950). Enamel protein was distinguished from other keratins by amino-acid 'spectra' plotted from paper chromatograms of their hydrolysates. However, the presence of enamel constituents reacting with Ehrlich's reagent (Hutton & Nuckolls, 1951) and with carbazole (Coolidge, 1951) has been announced. It is hoped that further information about these will be obtained when succeeding batches of enamel are demineralized by dialysis against neutralized ethylene diamine tetraacetic acid, which has been used in bone histology by Sreebny & Nikiforuk (1951).

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DEMONSTRATION

An Improved Flame Photometer. By L. LEYTON (introduced by E. C. FOULKES). (*Department of Forestry, University of Oxford*)

Some years' experience with the flame photometric technique has led to the design of a simple, inexpensive yet reliable model which possesses definite advantages over previous designs (e.g. Domingo & Klyne, 1949). Improvements have been effected by the following modifications. (1) The conventional 'right-angled' atomizer has been replaced by a more efficient concentric type which is easily cleaned or replaced. (2) The test solution is sprayed directly up a simple burner tube to the burner, thus ensuring that a high proportion actually reaches the flame. (3) A stainless steel burner has been introduced giving a spade-shaped flame and greater sensitivity. (4) A 'vernier' water manometer has been incorporated into the air system to allow more precise control of the air pressure. (5) Sintered-glass filters have been included in the air and acetylene streams to remove troublesome solid impurities. (6) Selectivity has been greatly increased by incorporating specific interference filters.

As a result of the greatly improved efficiency of the atomizer-burner unit, analyses can readily be made on as little as 0·5 ml. of test solutions of the following concentrations: K, 0·1; Ca, 0·5; Na, 0·02 μg./ml. These sensitivities have been obtained using a Cinema Television G.S. 18 photoelectric cell for potassium and an Eel selenium barrier layer cell for calcium and sodium, in conjunction with a Tinsley S.S. 6·45 galvanometer. For potassium, direct interference due to sodium radiation is negligible, for calcium it is of the order of 1%. Recovery tests show an overall accuracy of better than 5%. Full details of this instrument are shortly to be published (Leyton, 1951).

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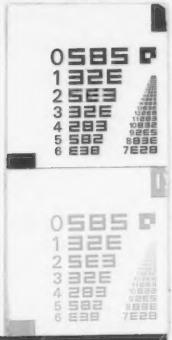
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